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AAO Foundation Final Report Form
(a/o 1/3/2018)

Type of Award
Biomedical Research Award

Name(s) of Principal Investigator(s)
F. Kurtis Kasper

Institution
The University of Texas Health Science Center at Houston

Title of Project
3D-Printed Nasoalveolar Molding Appliances for Cleft Lip and Palate

Period of AAOF Support
07-01-19 to 12-31-20
No Cost Extension 07-01-20 to 06-30-21

Amount of Funding
$29,851

Summary/Abstract
Objectives: Cleft lip and palate presents a considerable clinical burden globally. Pre-surgical management of cleft lip and palate patients with a nasoalveolar molding appliance can facilitate surgical repair of the cleft. However, standard techniques associated with the fabrication of nasoalveolar molding appliances are time- and labor-intensive. In addition, recent studies suggest that the early presence of an intraoral appliance can contribute to higher risk for caries in the cleft lip and palate patient population. Emerging technologies are enabling alternative approaches for the design and fabrication of nasoalveolar molding appliances, which harness computer-aided design and 3D printing to increase efficiency. While these technologies present the potential to advance the state-of-the-art of patient care through nasoalveolar molding, key properties associated with the materials applied in the fabrication of the appliances via 3D printing must be explored to evaluate the potential clinical utility of the approach. The overall objectives of the project were to evaluate the mechanical properties of acrylics applied in the fabrication of nasoalveolar molding (NAM) appliances using standard and 3D printing workflows and to characterize bacterial adhesion to the materials *in vitro*. Overall, this project provides a foundation of preliminary data regarding the potential clinical feasibility of 3D-printed acrylic nasoalveolar molding appliances for early management of cleft lip and palate and supports continued investigation of the emerging approach.

Specific Aim 1 – Investigation of the Adhesion of Soft Acrylic to Hard Acrylics Used in NAM Fabrication via Standard and 3D Printing Techniques: Specific Aim 1 involved investigation of the adhesion of soft acrylic to hard acrylics used in NAM fabrication via standard and 3D printing techniques. It was hypothesized that the mechanical properties of the 3D-printed materials (as reflected in tensile strength) would not differ significantly from standard cast hard acrylic, and that no significant differences would exist in the mechanical integrity of the bonding of soft acrylic to 3D-printed or standard cast hard acrylic. To this end, mechanical testing specimens (ASTM D638-14, Type IV) were prepared and applied to investigate the tensile strength of cast standard dental acrylic and 3D-printed acrylic (Dental LT Clear) (n=6/group). The samples were subjected to uniaxial tensile force at a cross-head speed of 5 mm/min until failure in an Instron Model 4465 universal testing machine. The ultimate tensile strength of the 3D-printed acrylic (78.98 ± 1.04 MPa) was statistically significantly greater than that of standard cast hard acrylic (41.54 ± 5.26 MPa; $P<0.001$). Accordingly, the hypothesis that there was no difference in the tensile strength between the two resins was rejected. The differences observed between the groups may reflect differences in the degree of curing between the cold-set cast hard acrylic and the light-cured 3D-printed acrylic, where the 3D-printed acrylic may present a higher degree of curing.

The tensile test method was then applied to investigate the adhesive strength of soft acrylic to the hard acrylics (standard cast acrylic and 3D-printed acrylic). An adaptation of the ASTM D638-14, Type IV test specimen was applied, in which a 3 mm span in the narrow section of the test specimen comprised soft acrylic, while the remainder of the specimen comprised hard acrylic. The following groups were investigated: (Group 1) cast standard dental acrylic, (Group 2) 3D-printed acrylic (Dental LT Clear), (Group 3) mechanically roughened 3D-printed acrylic, (Group 4) 3D-printed acrylic coated with a bonding agent, and (Group 5) mechanically roughened 3D-printed acrylic treated with a bonding agent. Specimens from each group were stored on the laboratory benchtop for 3.5 or 14 days at ambient temperature before testing (n=6/group/duration). The samples were subjected to uniaxial tensile force as described above until failure. The modes of failure were classified as cohesive failure within the soft acrylic or adhesive failure at the bonding interface between hard and soft acrylic. Groups 1 and 5 presented 100% cohesive failures in the soft acrylic at 3.5 and 14 days, while Group 2 presented 100% adhesive failures at 14 days (see Table I; Appendix). Only one adhesive failure was observed in Group 3, with a tensile strength of 0.44 MPa at 3.5 days. Group 4 presented a mixture of cohesive and adhesive failures at 3.5 and 14 days. Cohesive failure in the soft acrylic indicates that the strength of the adhesive interface exceeds the tensile strength of the soft acrylic. The hypothesis that the adhesive strength of soft acrylic to standard cast acrylic does not differ from that of 3D-printed acrylic was rejected under the conditions investigated. However, surface treatments of the 3D-printed acrylic can increase the adhesion of soft acrylic to 3D-printed acrylic to be comparable to the adhesion to standard cast acrylic, as reflected in cohesive failure rates. Specifically, the bonding of soft acrylic to 3D-printed acrylic increased with mechanical roughening of the hard acrylic prior to bonding, with or without the addition of a bonding agent, relative to untreated 3D-printed acrylic. The results suggest that surface treatment of the 3D-printed acrylic prior to bonding soft acrylic is warranted under the conditions investigated.

An additional study was completed using a peel test to compare the adhesive strength of soft acrylic bonded to 3D-printed acrylic versus bonding to standard hard acrylic. Specifically, rectangular 3D-printed acrylic samples were subjected to one of three different surface treatments: mechanical roughening, a chemical bonding agent, or a combination of mechanical roughening and a bonding agent (n=30/treatment). Control samples comprised soft acrylic bonded to standard cast hard dental acrylic (without treatment). Soft acrylic was applied to the samples and cured at ambient temperature for 3.5, 7, or 14 days (n=10/duration). The samples were tested with an Instron Model 4465 universal testing machine using a 180-degree peel test adapted from the ASTM D903 standard test method. Surface treatment of 3D-printed samples with a bonding agent or a combination of mechanical roughening and a bonding agent did not increase the mechanical strength of the samples relative to the control group under the conditions investigated (see Table II; Appendix). Curing duration did not appear to have an effect on mechanical strength. Overall, the adhesive strength of soft acrylic to 3D-printed acrylic depends upon the surface treatment method employed, with mechanical roughening providing the greatest adhesion (as reflected in cohesive failure of the soft acrylic). However, additional investigation is warranted to characterize bonding of soft acrylic to 3D-printed acrylic for NAM appliance fabrication.

A follow-up study investigated the effect of post-print ultraviolet light curing time on the bond strength of soft acrylic to 3D-printed hard acrylic. It was hypothesized that the curing duration would significantly affect the bond strength of the soft acrylic to the 3D-printed acrylic, with longer durations resulting in lower bond strengths. Rectangular 3D-printed acrylic samples were subjected to one of four different post-print ultraviolet light curing treatments: no post-print curing, 10 minutes curing at 80^{o}C, 20 minutes curing at 80^{o}C (manufacturer recommendation), and 40 minutes curing at 80^{o}C (n=16/treatment). Control samples comprised soft acrylic bonded to standard cast hard dental acrylic (without treatment). Soft acrylic was applied to the samples and cured at ambient temperature for 12-24 hours prior to testing with an Instron Model 4465 universal testing machine using a 180-degree peel test adapted from the ASTM D903 standard test method. The standard acrylic samples presented 100% cohesive failures, indicating that the bond strength of the soft acrylic to the standard acrylic was greater than the tear strength of the soft acrylic. The standard acrylic group had a significantly higher bond strength than all of the 3D-printed acrylic groups ($P<0.05$; see Table III; Appendix). An adhesive mechanism of failure was observed in 100% of the 3D-printed samples. There was no significant difference in bond strength between the 10, 20, and 40-minute cure groups ($P>0.05$). However, the no cure group (mean maximum load 7.76 ± 0.45 N) had a significantly higher bond strength than the 10-, 20-, or 40-minute groups ($P<0.05$). The higher bond strength for the no cure group may reflect the presence of residual reactive groups on the surface of the 3D-printed samples that contribute to chemical bonding with the soft acrylic, and the availability of these groups may decrease with post-print ultraviolet light curing.

Additional work was completed to investigate if the use of a primer would affect the bond strength of the soft acrylic to mechanically-roughened 3D-printed acrylic. Rectangular 3D-printed acrylic samples were prepared as before, and the 20-minute post-print curing duration was applied. The printed samples were mechanically roughened and divided into two groups, with one group treated with a primer before the addition of soft acrylic, while primer was not applied in the remaining group (n=25/treatment). Soft acrylic was applied to the samples and cured at ambient temperature for 12-24 hours prior to testing with an Instron Model 4465 universal testing machine using a 180-degree peel test adapted from the ASTM D903 standard test method. Two of the 25 samples in the roughened plus primer group exhibited cohesive failures, while the rest of the samples in both groups presented an adhesive mechanism of failure. There was no significant difference in bond strength between the roughened only group and the roughened plus primer group (16.66 ± 0.44 N and 17.59 ± 0.043 N, respectively; $P>0.10$). Surface roughness measurements using a surface profilometer found no significant difference in the surface roughness between the roughened only group and the roughened plus primer group ($P>0.10$).

Specific Aim 2 – Investigation of Bacterial Adhesion to NAM Appliance Acrylics: Specific Aim 2 involved investigation of the adhesion of certain pathogenic bacterial strains to samples of the hard and soft acrylics investigated in Specific Aim 1. It was hypothesized that bacterial adhesion to 3D-printed and standard cast hard acrylic surfaces under culture *in vitro* would not significantly differ. A total of 150 sample discs were fabricated from standard hard acrylic, soft acrylic, and 3D-printed acrylic (n=50/material). The surfaces of the hard acrylic and 3D-printed samples were polished on one side and left as cast/printed on the opposite side. All three sample types were bathed in bacterial biofilm media for 24 hours. A bacterial adhesion assay was completed to quantify bacterial adhesion to the samples (see Figure 1). Additionally, surface roughness was measured with a surface profilometer on all sample types, including polished and unpolished surfaces. The results of this study show that bacterial species *E. coli*, *S. aureus*, *Klebsiella*, and *S. epidermidis* have a propensity for higher bacterial growth on traditional hard acrylics versus 3D-printed acrylics when assessing total biofilm signals (see Figure 2). The exception to this among the bacteria studied is *S. pneumoniae,* which had higher total biofilm signals on 3D-printed acrylic when compared to cast standard hard and soft acrylic alone. Interestingly, soft acrylic generally proved to harbor less bacteria than traditional hard acrylics and 3D-printed acrylics. Polishing of the surfaces of the hard acrylic samples reduced the surface roughness values (Ra) from 2.31 µm to 1.25 µm for the standard hard acrylic and from 2.18 µm to 0.82 µm for the 3D-printed acrylic. However, polishing of the surfaces of the 3D-printed and hard acrylic samples had no statistically significant effect on bacterial adhesion except in the case of *E. coli*, in which the bacteria strongly favored polished hard acrylic surfaces. The soft acrylic samples measured an average Ra of 3.62 µm. The data suggest that 3D-printed acrylics generally offer an advantage over traditional hard acrylics commonly used in the fabrication of NAM appliances in terms of harboring less bacteria. Polishing the surfaces of the acrylics is generally not beneficial in reducing bacterial load under the studied conditions.

1. Were the original, specific aims of the proposal realized?

Yes

2. Were the results published?

a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers

1. Kasper FK, Ghivizzani M, Chiquet BT. “3D Printing for the Fabrication of Nasoalveolar Molding (NAM) Appliances to Facilitate Repair of Cleft Lip and Palate: A Narrative Review.” Journal of 3D Printing in Medicine, 3(4):195-208, 2019. (DOI: 10.2217/3dp-2019-0019)

2. Ghivizzani M. Proof of Concept and Mechanical Properties for a Digitally Designed 3D-Printed Nasoalveolar Molding Appliance for Unilateral Cleft Lip and Palate. The University of Texas School of Dentistry at Houston, ProQuest Dissertations Publishing, 2019. 13903319.

b. Was AAOF support acknowledged?

Yes, as appropriate, AAOF support was acknowledged in each publication.

c. If not, are there plans to publish? If not, why not?

Yes, the project involved contributions from four residents in partial completion of the requirements of the degree of Masters of Science in Dentistry. Some project results were published in a thesis detailed above and other results will be published in planned theses, as follows:

1. Garza J. “Bacterial Adhesion to Acrylics for Nasoalveolar Molding Appliance Fabrication,” Masters of Science in Dentistry Thesis, Department of Orthodontics, The University of Texas School of Dentistry at Houston, Houston, Texas. (in preparation).

2. Wehr C. “3D Printed Fabrication of Nasoalveolar Molding Appliance in Cleft Lip and Palate Patients from MRI Face Scan,” Masters of Science in Dentistry Thesis, Department of Pediatric Dentistry, The University of Texas School of Dentistry at

Houston, Houston, Texas. (in preparation)

3. Willis N. "The Effect of Post-Print Processing Protocol on Adhesive Strength of Soft Acrylic to 3D-Printed Hard Acrylic," Masters of Science in Dentistry Thesis, Department of Orthodontics, The University of Texas School of Dentistry at Houston, Houston, Texas. (in preparation).

In addition, three research manuscripts based on the results of the project are presently under preparation for submission to peer-reviewed journals. In each case, the submissions will acknowledge AAOF support, as appropriate.

3. Have the results of this proposal been presented?

a. If so, list titles, author or co-authors of these presentation/s, year and locations

1. "Emerging Applications of 3D Printing in Orthodontics," Kasper FK*. Department of Diagnostic and Biomedical Sciences Research Seminar Series, The University of Texas School of Dentistry at Houston, Houston, Texas. (June 14, 2021) (via WebEx)

2. "Current and Future Applications of 3D Printing in Orthodontics," Kasper FK*. American Association of Orthodontists, Society of Educators, Webinar. (May 21, 2021)

3. "3D Printed Fabrication of Nasoalveolar Molding Appliance in Cleft Lip and Palate Patients from Digital MRI Face Scan," Wehr C*, Chiquet B, Acharya B, Greives M, Cardenas A and Kasper FK. American Academy of Pediatric Dentistry Annual Meeting, Boston, Massachusetts. (May 20-30, 2021) (Meeting Held Virtually)

4. "3D Printed Fabrication of Nasoalveolar Molding Appliance in Cleft Lip and Palate Patients from MRI Face Scan," Wehr C*. Master of Science in Dentistry Thesis Defense, Department of Pediatric Dentistry, The University of Texas School of Dentistry at Houston, Houston, Texas. (May 5, 2021) (via WebEx)

5. "Current and Emerging Applications of 3D Printing in Orthodontics," Kasper FK*. Alignment Club, The University of Texas School of Dentistry at Houston, Houston, Texas. (September 29, 2020) (via WebEx)

6. "Leaving the Stone Age: Applying Biomaterials and 3D Printing to Meet Clinical Needs," Kasper FK*. Rutgers School of Dental Medicine 2020-2021 Research Seminar Series, Newark, New Jersey. (September 8, 2020) (via WebEx)

7. "Current and Emerging Applications of 3D Printing in Orthodontics, Restorative, and Regenerative Dentistry," Kasper FK* and Bertassoni LE*. 2020 Society For Biomaterials Webinar Series, Webinar. (July 29, 2020)

8. "Evidence-based Guidance for 3D Printing Applications in Clear Aligner Therapy," Kasper FK*. The Houston Center for Biomaterials and Biomimetics (HCBB), The University of Texas School of Dentistry at Houston, Houston, Texas. (July 9, 2020) (via WebEx)

9. "Soft Acrylic Adhesion to 3D-Printed Acrylic for Nasoalveolar Molding Applications," Crell B*, Chiquet BT, Kasper FK. 2019 UTSD Student Research

Showcase, The University of Texas School of Dentistry at Houston, Houston, Texas. (October 29, 2019) [Best Poster Presentation Award – Biomaterials and Clinical Methods Category]

10. "Evidence-based Guidance for 3D Printing Applications in Dentistry," Kasper FK*. Greater Houston Dental Society, Tale and Ale Program, Braeburn Country Club, Houston, Texas. (September 24, 2019)

b. Was AAOF support acknowledged?
Yes, in each presentation

c. If not, are there plans to do so? If not, why not?
The results will continue to be included in presentations, as appropriate, with proper acknowledgement of support from AAOF for the work.

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
As a bioengineer, I am thrilled to explore exciting new frontiers at the intersection of engineering and orthodontics, and funding from the AAOF has been vital to enable my investigations in these areas that traditionally are not targets for funding from federal sources. The funding from AAOF provides me with opportunities to expand my exposure to the challenges of clinical orthodontics, to collaborate with clinicians and researchers in the field, to increase my research profile, and to broaden my professional network. The benefits enabled by AAOF support provide a firm foundation upon which I plan to continue to build my research program in topics of relevance to orthodontics.

Table I. Uniaxial tensile adhesive testing of soft acrylic bonded to standard cast dental acrylic (Group 1), 3D-printed acrylic (Group 2), mechanically roughened 3D-printed acrylic (Group 3), 3D-printed acrylic treated with a bonding agent (Group 4), and mechanically roughened 3D-printed acrylic treated with a bonding agent (Group 5).

Test Group	Days	Number (Percent)	Adhesive Failures Mean Tensile Strength (MPa)	Standard Deviation (MPa)	Cohesive Failures Number (Percent)
Group 1	3.5 14	0 (0) 0 (0)	n/a n/a	n/a n/a	6 (100) 6 (100)
Group 2	3.5 14	2 (33.3) 6 (100)	0.10 0.16	0.04 0.04	4 (66.7) 0 (0)
Group 3	3.5 14	1 (16.7) 0 (0)	0.44 n/a	n/a n/a	5 (83.3) 6 (100)
Group 4	3.5 14	4 (66.7) 3 (50)	0.11 0.17	0.02 0.03	2 (33.3) 3 (50)
Group 5	3.5 14	0 (0) 0 (0)	n/a n/a	n/a n/a	6 (100) 6 (100)

Table II. Adhesive peel testing of soft acrylic bonded to standard cast dental acrylic (Group 1), mechanically roughened 3D-printed acrylic (Group 2), 3D-printed acrylic treated with a bonding agent (Group 3), and mechanically roughened 3D-printed acrylic treated with a bonding agent (Group 4).

Test Group	Days	Number (Percent)	Adhesive Failures Mean Maximum Load (N)	Standard Deviation (N)	Cohesive Failures Number (Percent)
Group 1	3.5	0 (0)	n/a	n/a	10 (100)
	7	0 (0)	n/a	n/a	10 (100)
	14	0 (0)	n/a	n/a	10 (100)
Group 2	3.5	1 (10)	18.20	n/a	9 (90)
	7	2 (20)	15.89	1.76	8 (80)
	14	2 (20)	16.27	2.99	8 (80)
Group 3	3.5	10 (100)	1.86	2.36	0 (0)
	7	10 (100)	2.49	1.38	0 (0)
	14	10 (100)	2.29	1.29	0 (0)
Group 4	3.5	10 (100)	9.53	3.43	0 (0)
	7	10 (100)	4.87	3.91	0 (0)
	14	10 (100)	8.06	4.12	0 (0)

Table III. Adhesive peel testing of soft acrylic bonded to standard cast dental acrylic (Group 1) and 3D-printed acrylic post-cured with exposure to ultraviolet light for 0 minutes (Group 2), 10 minutes (Group 3), 20 minutes (Group 4), or 40 minutes (Group 5) at 80^{o}C.

Test Group	Adhesive Failures Mean Maximum Load (N)	Standard Error (N)	Cohesive Failures Number (Percent)
Group 1	21.75	0.62	16 (100)
Group 2	7.76	0.45	0 (0)
Group 3	4.67	0.27	0 (0)
Group 4	5.56	0.44	0 (0)
Group 5	5.10	0.78	0 (0)

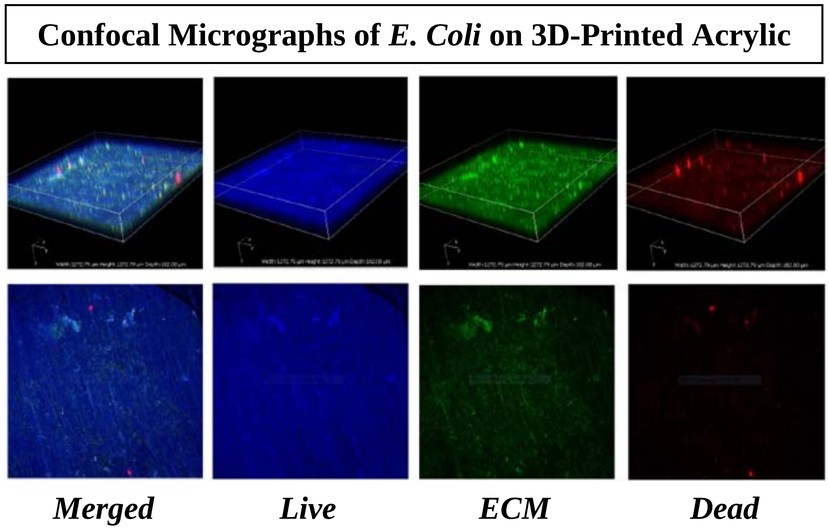

Figure 1. Representative confocal micrographs showing live bacteria (blue), extracellular material (ECM; green), and dead bacteria (red).

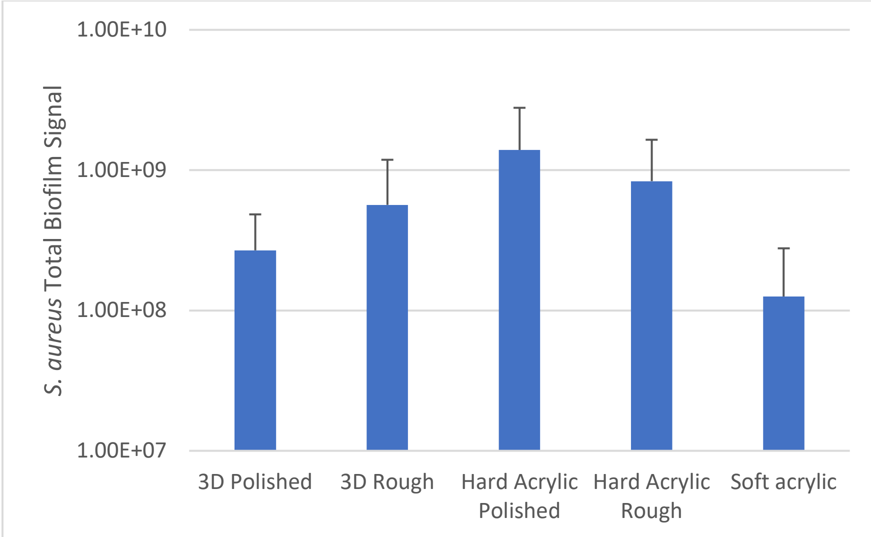

Figure 2. Representative data showing *S. aureus* total biofilm signal following 24 hours of culture on polished and unpolished/rough 3D-printed acrylic, polished and unpolished/rough standard hard acrylic, and soft acrylic. Significant differences were observed between 3D Polished and Hard Acrylic Polished (P=0.04) and between Hard Acrylic Polished and Soft Acrylic (P=0.02).