

ABSTRACT

Objectives: To determine the best surface treatment for bonding 3D-printed crown materials with different compositions.

Methods: Two different 3D printed crown materials (Crown and Ceramic Crown, SprintRay) with different compositions were examined. 4mm thick blocks of the 2 materials were printed in a DLP printer (Pro55S, SprintRay) and cured according to IFU in a curing unit (ProCure 2, SprintRay). Specimens were mounted flat in acrylic with their “intaglio” surfaces untouched. Half the specimens from each group were sandblasted with 50-micron alumina (2 bar pressure). Then specimens were divided into 4 groups (n=10): Gr1 – no further treatment; Gr2-1 coat of silane (Porcelain Primer, Bisco); Gr3 – 1 coat of universal adhesive (Scotchbond Universal, 3M); Gr4 – 1 coat of silane then 1 coat of universal adhesive. Specimens were placed into an Ultradent shear bond strength apparatus in which a plastic cylindrical mold was filled with Filtek Supreme composite and light cured (20sec, Elipar S10, 3M, 1100mW/cm²). After removing the mold, the specimens were stored for 8 weeks in 37C water. The specimens were debonded with a circular notched-edge blade applied at 1mm/min and shear bond calculated. The data were compared with a 2-way ANOVA (factors: sandblasting and primer) and Tukey post-hoc analysis for both materials independently, p<0.01 considered meaningful. The composition of the materials were compared by determining resin content using FTIR and filler content by thermogravimetric analysis.

Results: For both materials, factors sandblasting and primer were significant (p<.01) but their interaction was not (p=.43 for Crown and p=.34 for Ceramic Crown). Sandblasting improved the bond strength for both materials. The Tukey post-hoc analysis grouped primer treatments into the same statistically different groups for both materials: Gr1 and Gr2 < Gr3 and Gr4. Filler percentage of Crown was 32.7% and Ceramic Crown was 48.2%. Resin content was similar for both materials.

Conclusions: The most effective method to bond to 3D printed crowns (regardless of filler percentage) was to sandblast with 50-micron alumina and apply a layer of adhesive (with or without previous application of silane).

INTRODUCTION

3D printed crown materials are commonly used for temporary restorations but may be used as permanent restorations if their filler content is greater than 50% (according to ADA).[1] Several manufacturers produce crown resins with filler below 50% that may be used for temporary crowns and crown resins with >50% filler that may be used for permanent crowns.[2] The advantage of 3D printed crowns is that they may be manufactured at a lower cost than ceramic crowns due to the decreased cost of equipment as well as the resin material.[3] Additionally, multiple crowns may be printed at the same time. If these crowns are used as permanent crowns or long-term temporary crowns, a dentist may choose to bond the crowns to the tooth using resin cement. The method of preparing the surface of the crown for bonding is important to increase its bond. When bonding to resin composite materials, etching with hydrofluoric acid is not recommended and surface roughening should be performed with sandblasting.[4] When chemically priming the crown materials, chemical bonding may be performed to the included resin the material or the glass-based fillers. If bonding to the methacrylate resins in the 3D printed resins, using a methacrylate based bonding agent could be used. If bonding to the glass-based fillers, silane could be used. The purpose of this study is to determine: 1. if sandblasting improves bond strength to 3D printed crown resins, 2. if the use of bonding agent and/or silane improves the bond to 3D printed crown resins, 3. if the type of 3D printed crown material (temporary vs permanent) affects which surface treatment produces the highest bond strength.

MATERIALS AND METHODS



Crown, SprintRay
Temporary material



Ceramic Crown, SprintRay
Permanent material

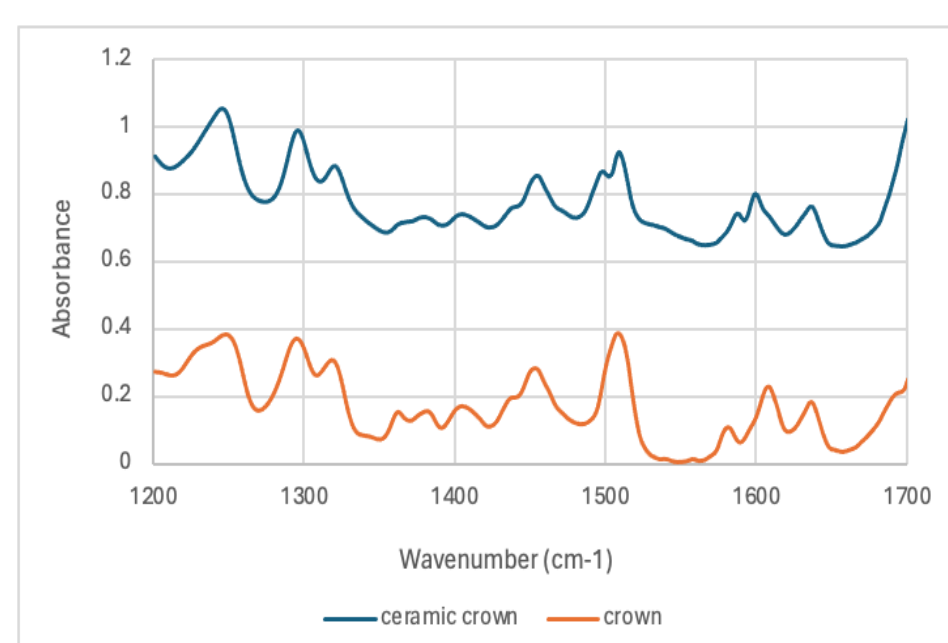


Scotchbond Universal+, 3M
Universal adhesive

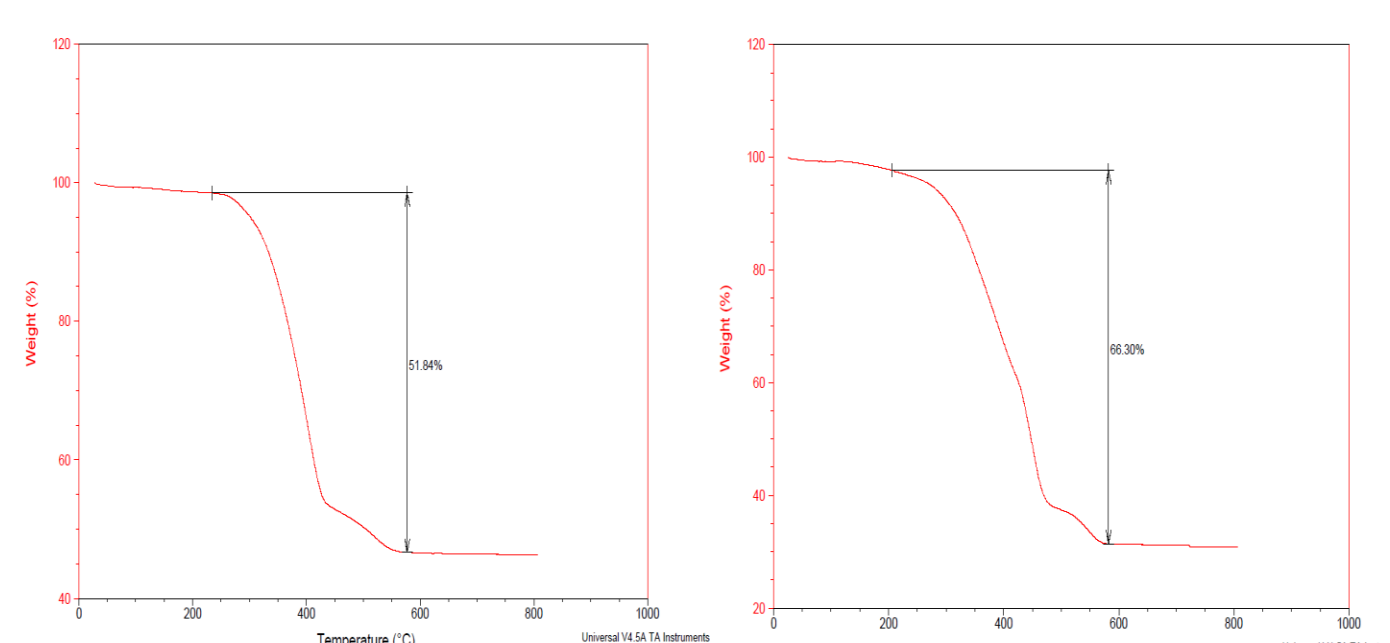


Porcelain primer, Bisco
Silane primer

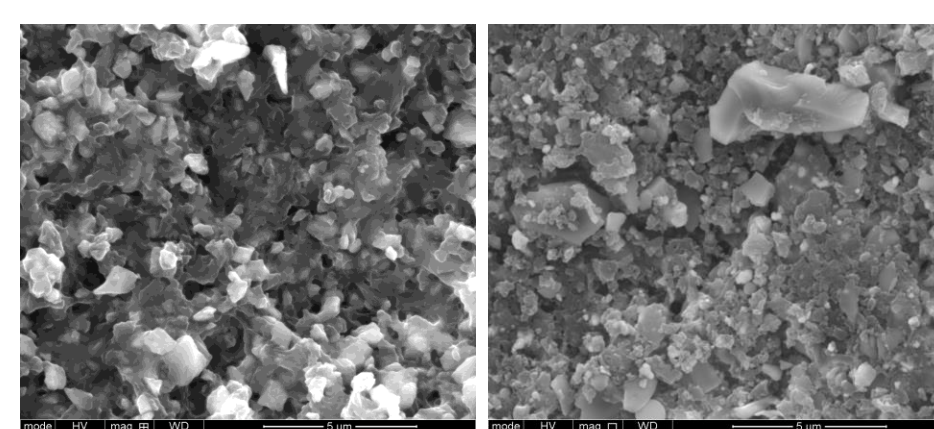
In order to determine filler weight percentage, a sample of each material was analyzed by thermogravimetric analysis. Approximately 15mg of material was inserted into a thermogravimetric analysis device (TG50, Mettler Toledo) and the test was run at a rate of 10° C/min until a consistent mass was achieved. The remaining ash was examined under SEM. In order to determine resin composition, the resins were analyzed with Fourier transform infrared (FTIR) spectroscopy using an Alpha II ATR-FTIR Spectrophotometer (Bruker). The spectra were collected at 400-4,000 cm⁻¹ wavelength range with 16 sample scans and 16 background scans at a resolution of 4 cm⁻¹. Spectra were baseline corrected in OPUS, min-max normalized, and displayed with a vertical offset for ease of viewing.



FTIR show a similar spectra for Crown and Ceramic crown which implies a similar resin for both materials

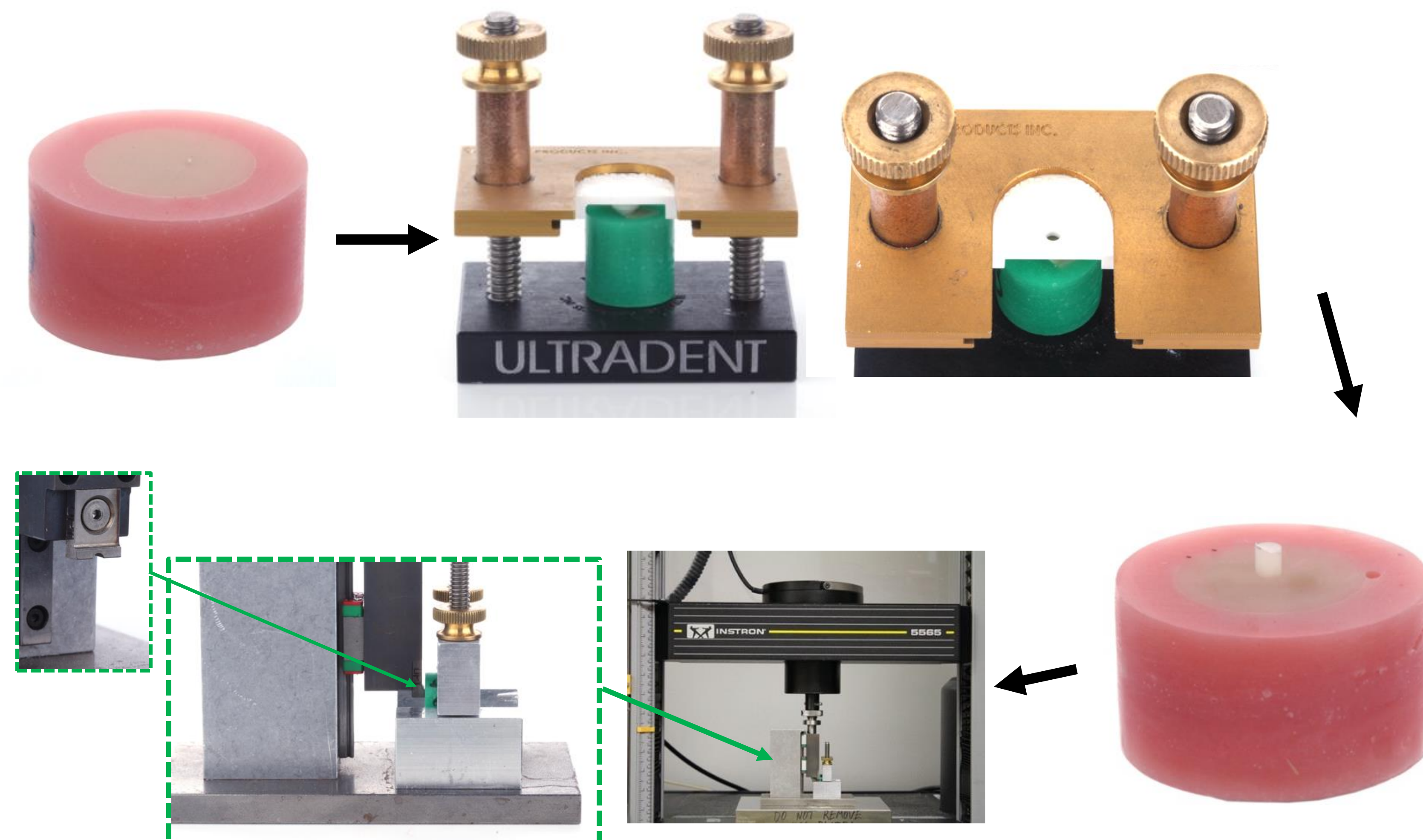


Thermogravimetric analysis reveals a 48.2% filler weight for Ceramic Crown and a 32.7% filler weight for Crown.



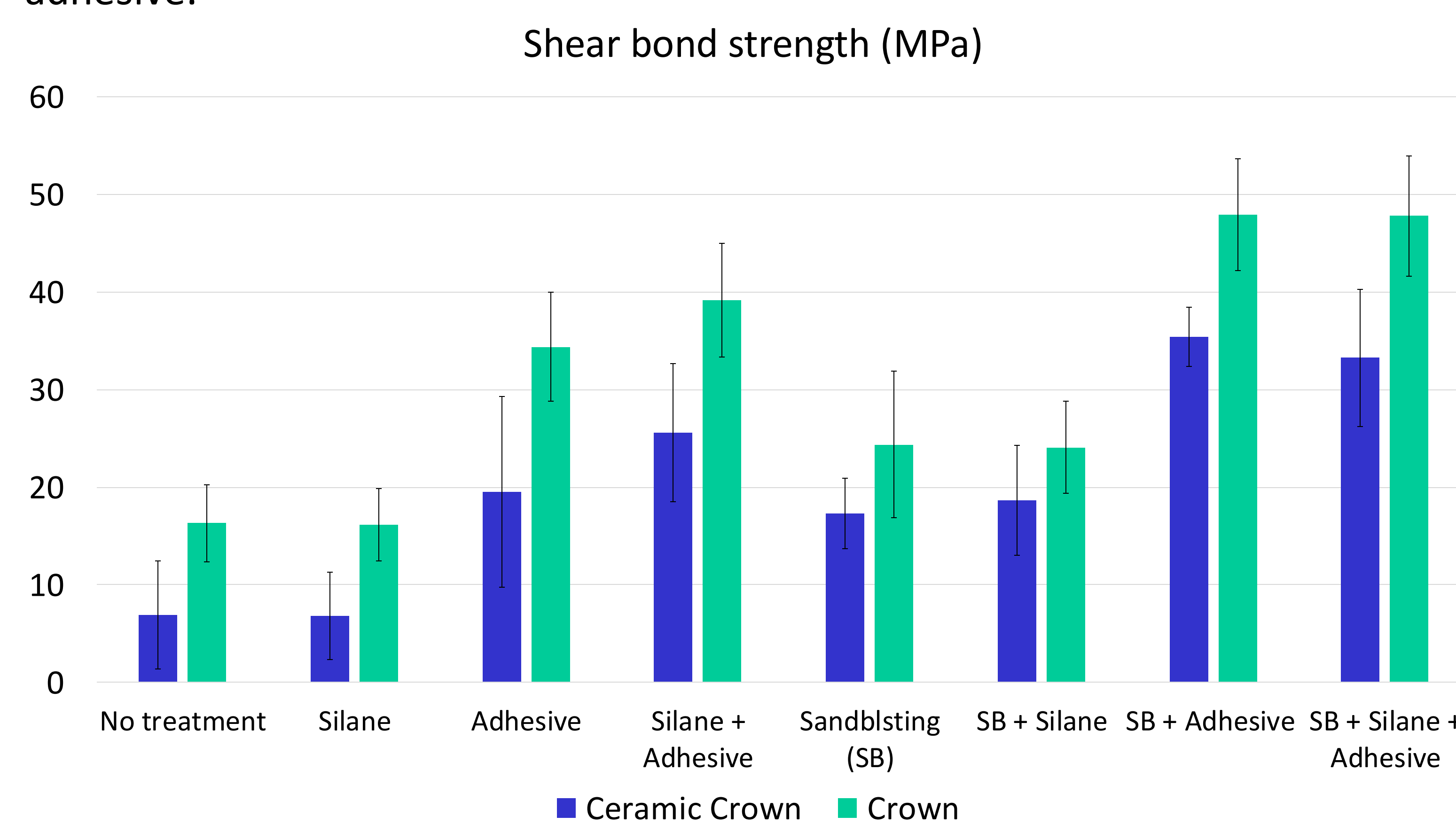
Analysis of filler in Crown reveals Si, Ba, Al and O whereas the filler in Ceramic Crown is Si and O. This suggests a barium aluminasilicate glass in Crown and silica glass in Ceramic Crown

4mm thick blocks of the 2 materials were printed in a DLP printer (Pro55S, SprintRay) and cured in according to IFU in a curing unit (ProCure 2, SprintRay). Specimens were mounted flat in acrylic with their “intaglio” surfaces untouched. Half the specimens from each group were sandblasted with 50-micron alumina. Then specimens were divided into 4 groups (n=10): Gr1 – no further treatment; Gr2 – 1 coat of silane (Porcelain Primer, Bisco); Gr3 – 1 coat of universal adhesive (Scotchbond Universal, 3M); Gr4 – 1 coat of silane then 1 coat of universal adhesive. Specimens were placed into an Ultradent shear bond strength apparatus in which a plastic cylindrical mold was filled with Filtek Z250 composite and light cured (20sec, Elipar S10, 3M, 1100mW/cm²). After removing the mold, the specimens were stored for 8 weeks in 37C water. The specimens were debonded with a circular notched-edge blade applied at 1mm/min and shear bond calculated. The data were compared with a 1-way ANOVA and Tukey post-hoc analysis, p<0.01 considered meaningful.



RESULTS

For both materials, factors sandblasting and primer were significant (p<0.01) but their interaction was not (p=.43 for Crown and p=.34 for Ceramic Crown). Sandblasting improved the bond strength for both materials. The Tukey post-hoc analysis grouped primer treatments into the same statistically different groups for both materials: no treatment and silane < adhesive and silane + adhesive.



CONCLUSIONS

The most effective method to bond to 3D printed crowns (regardless of filler percentage) was to sandblast with 50-micron alumina and apply a layer of adhesive (with or without previous application of silane).

REFERENCES

- ada.org/publications/cdt/glossary-of-dental-clinical-terms#porc
- Daher R, Ardu S, di Bella E, et al. Efficiency of 3D-printed composite resin restorations compared with subtractive materials: Evaluation of fatigue behavior, cost, and time of production. J Prosthet Dent., 2022;1:S0022-3913(22)00481-4.
- Bora PV, Sayed Ahmed A, Alford A, Pittman K, Thomas V, Lawson NC. Characterization of materials used for 3D printing dental crowns and hybrid prostheses. J Esthet Restor Dent. 2024 Jan;36(1):220-230. doi: 10.1111/jerd.13174. Epub 2023 Nov 26. PMID: 38008797.
- Nejat AH, Lee J, Shah S, Lin CP, Kulkarni P, Chavali R, Lawson NC. Retention of CAD/CAM resin composite crowns following different bonding protocols. Am J Dent. 2018 Apr;31(2):97-102. PMID: 29630794; PMCID: PMC7135944.



Mandibular Fibrous Dysplasia With Maxillary Osteonecrosis – Diagnosis And Rehabilitation



Janhavi, Main BDS,^{1,3,7,8} Anmol Gupta, DDS,^{2,3,7,8} Steven R. Singer, DDS,^{4,6,7,8} Adriana G. Creanga, DMD, MS^{5,6,7,8} Gayathri Subramanian, PhD, DMD^{7,8}

¹Student, ²General Practice Resident, ³Equal contributors, ⁴Chair and Division Director, ⁵Assistant Professor, ⁶Division of Oral and Maxillofacial Radiology

⁷Department of Diagnostic Sciences, ⁸Rutgers School of Dental Medicine, Newark, NJ

Fibrous Dysplasia- spectrum Developmental Disorder

Fibrous dysplasia (FD) is a non-hereditary, developmental skeletal disorder affecting one (monostotic) or multiple bones (polyostotic). It can be attributed to a mutation in the GNAS1 gene. Craniofacial involvement is seen in 10% of monostotic and at least 50% of polyostotic instances. FD commonly manifests with facial asymmetry. Non-McCune-Albright FD lesions usually present in the second decade of life and stop growing at the end of puberty. Premature surgical resection may risk an exuberant recurrence.

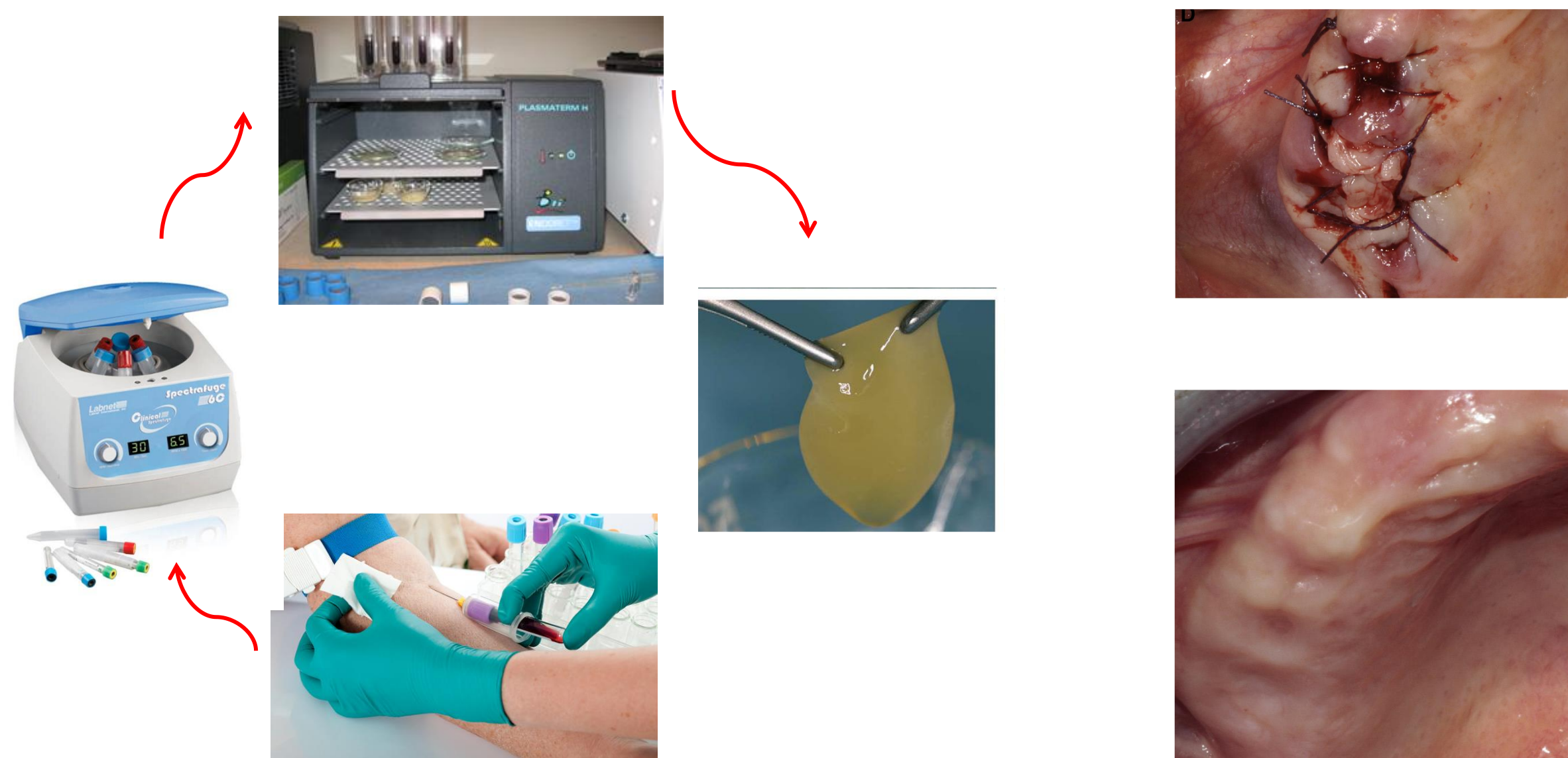
Patient History

A 72-year-old patient presented to the GPR clinic from the UG clinic for an upper complete and lower partial denture. The patient had a history of a large bony lesion on the posterior right side since age 14, previously diagnosed as ossifying fibroma. He reported a history of surgical resection of mandibular FD as a youth, followed by lesion recurrence. No further intervention was advised at that time. Present symptoms included buccolingual expansion, chronic periapical abscess near tooth 28, and bony spicules in the upper anterior ridge. CBCT was recommended to assess the lesions in the anterior maxilla and right mandible.

Unique Diagnostic Considerations

Three aspects of his assessment and management were notable. 1) While the radiographic changes from FD were extensive, they were remarkably similar to those evident on a panoramic radiograph taken over 13 years ago; 2) FD is not known to increase the risk for osteonecrosis in the absence of treatment with antiresorptive medications. To mitigate further risk for osteonecrosis and optimize soft and hard tissue healing following residual root removal and alveoplasty, a conservative treatment approach utilizing Plasma-Rich-in-Growth-Factors was implemented following careful debridement of the necrotic area. 3) A biopsy-proven definitive diagnosis of neither the lesion in the anterior maxilla nor the mandibular lesion was pursued, given the potential risk for post-procedure osteonecrosis and subsequent pathological fracture, given the extensive FD lesion.

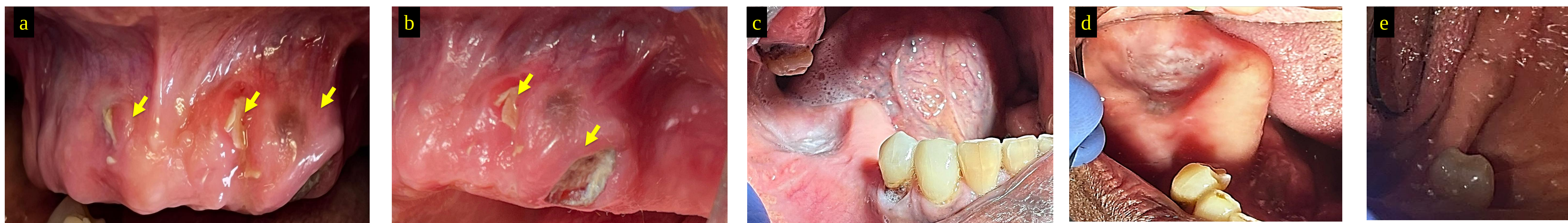
Dental Management and Rehabilitation



- Dental Rehabilitation**
 - Plasma Rich in Growth Factors (PRGF)-assisted extraction of teeth #2, 11, and #17 and alveoplasty and debridement of osteonecrotic bone
 - Endodontic treatment of #28, restoration of carious lesions
 - Enameloplasty to optimize the lower occlusal plane
 - Complete maxillary denture on the optimized occlusal plane
- Definitive Diagnosis**
 - Genetic Testing for FD from blood/saliva may help but may yield a false-negative result if the post-zygotic mutation is only detectable in the affected bone
 - A hard-tissue biopsy may help, but it is not an option with this patient

Clinical Presentation –Expanded Mandibular Bone with Maxillary Osteonecrosis

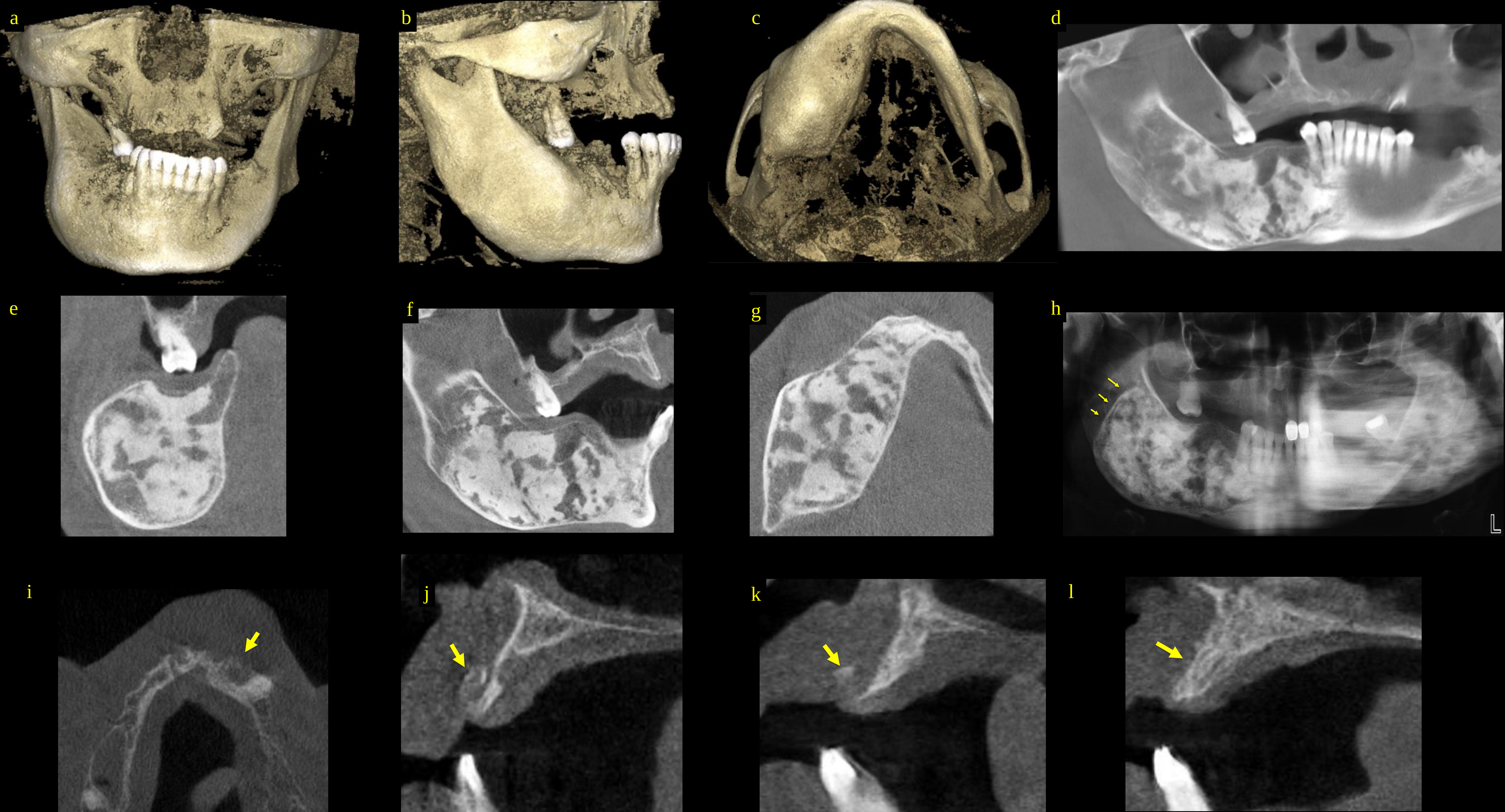
- Maxillary osteonecrosis (Figures a, b)
- Residual roots #11, 17 (Figures c, e)
- Non-restorable #2 (Figure c)
- Buccal protuberance (a), will require alveoplasty
- #21, 28 caries, buccal fistula near nonvital #28
- Occlusal canting evident in mandibular plane
- Expansion of right mandible, saucerization by #2 (d)
- Intact mucosal tissue over the lesion (d)
- No neurological deficit involving inferior alveolar nerve



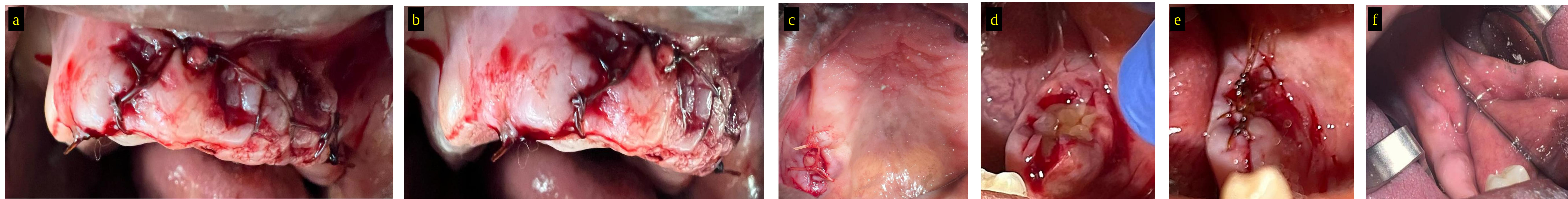
Diagnosis: Fibrous Dysplasia vs Ossifying Fibroma, Developmental or Tumor?

Radiographic appearance of the maxillary and mandibular lesions

- Multi-directional bone growth and expansion without perforation (Figures a, b, c)
- Adjusted mid-coronal and sagittal (Figures e, f) and axial (Figure g) demonstrating bone growth in the right mandible.
- Radiographic description: Severe asymmetry caused by bone expansion in all directions, confined within the expanded cortices. Mixed-density lesion with alternating low-density and “ground glass” high-density patches.
- Earlier conventional panoramic radiograph exhibiting facial asymmetry with bone enlargement in the right mandibular body and ramus, a heterogeneous radiopaque-radiolucent entity displaying altered/remodeled trabecular pattern and what appeared to be a well-defined corticated border (arrows, Figure h)- led to the initial diagnosis of ossifying fibroma in 2013.
- Current Cone-beam CT reconstructed panoramic view (Figure d) demonstrates that the lesion is poorly demarcated and blends gradually into the unaffected surrounding bone. Expansion is seen, with no perforation. Revised diagnosis: Monostotic fibrous dysplasia.
- Axial (Figure i) & sagittal (j, k, l) slices help to correlate with clinical and intra-operative images for anterior maxilla osteonecrosis– altered trabecular pattern, detached bone fragments in the vicinity of poorly healing extraction sockets.



Treatment: PRGF-assisted Dental Extractions, Surgical Debridement of Osteonecrosis, Alveoplasty, and Wound Closure



Views of the PRGF-assisted post-surgical wound closure in maxillary arch following debridement of osteonecrosis, extraction of #11, alveoplasty (Figures a, b), extraction site of #2 (Figure c), #17 (Figure d, e), preliminary healing of wound site #17. #28 is being endodontically treated, #21 and 28 carious lesions were treated and restored with glass ionomer. Mandibular occlusal plane was optimized with enameloplasty. Patient is being optimized for maxillary complete denture fabrication.

Enhancing Collaboration: A Digital Communication Protocol for General Dentists and Orthodontists

Natalie Pesun¹, Sadaf Salehi Qeshmi¹, Lisa Xu¹, Steve Ruiz², Afroditi Pita²

¹Post-graduate Resident, Advanced Education in General Dentistry, University of Connecticut Health Center

²Assistant Professor, General Dentistry Department, University of Connecticut Health Center

INTRODUCTION

Demand for orthodontic treatment and implants in adults has increased over the past years. This is in part due to increased public knowledge regarding benefits of enhancing dental alignment (1). Many of the adult patients seeking to replace missing or compromised teeth with implants benefit from orthodontic treatment to better manage the spaces, which necessitates a combined orthodontic-restorative approach (2). Conventionally, methods such as written or verbal descriptions of desired movements and average measurements have been used to communicate desired outcomes to orthodontists. This poster explores a digital design approach to create an ideal simulated outcome of proposed orthodontic and restorative treatments as a method to facilitate communication between the restorative dentist and orthodontist and enhance patient motivation.

MATERIALS AND METHODS

1. An intra-oral scan of the patient was obtained
2. The scan was uploaded into exocad, and the parameters were defined:
 - For existing teeth that are planned for movement:
 - Anatomic pontic- virtual extraction
 - For missing teeth that are to be waxed-up:
 - Anatomic pontic- no virtual extraction
 - The option to load a pre-op scan was selected to aid in design and allow for comparison.
3. In the design module, the existing teeth were defined and virtually extracted as direct copies to allow for their movement within the design module (Figure 1).
4. The spaces for the missing teeth were defined and virtual teeth were populated by the software.
5. The existing teeth were moved into the desired positions without modifying the size or shape.
6. A wax up was created for the missing teeth to match the patient's existing dentition and simulate the final restorative outcome.
7. The gingiva was then shaped to simulate the anticipated changes to the soft tissue with orthodontic movements.
8. The changes were compared to the initial scans to assess the amount of movement being requested (Figure 2).
9. Cut view was used to take measurements of the simulation in the edentulous spaces to be provided to the orthodontist (Figure 3).

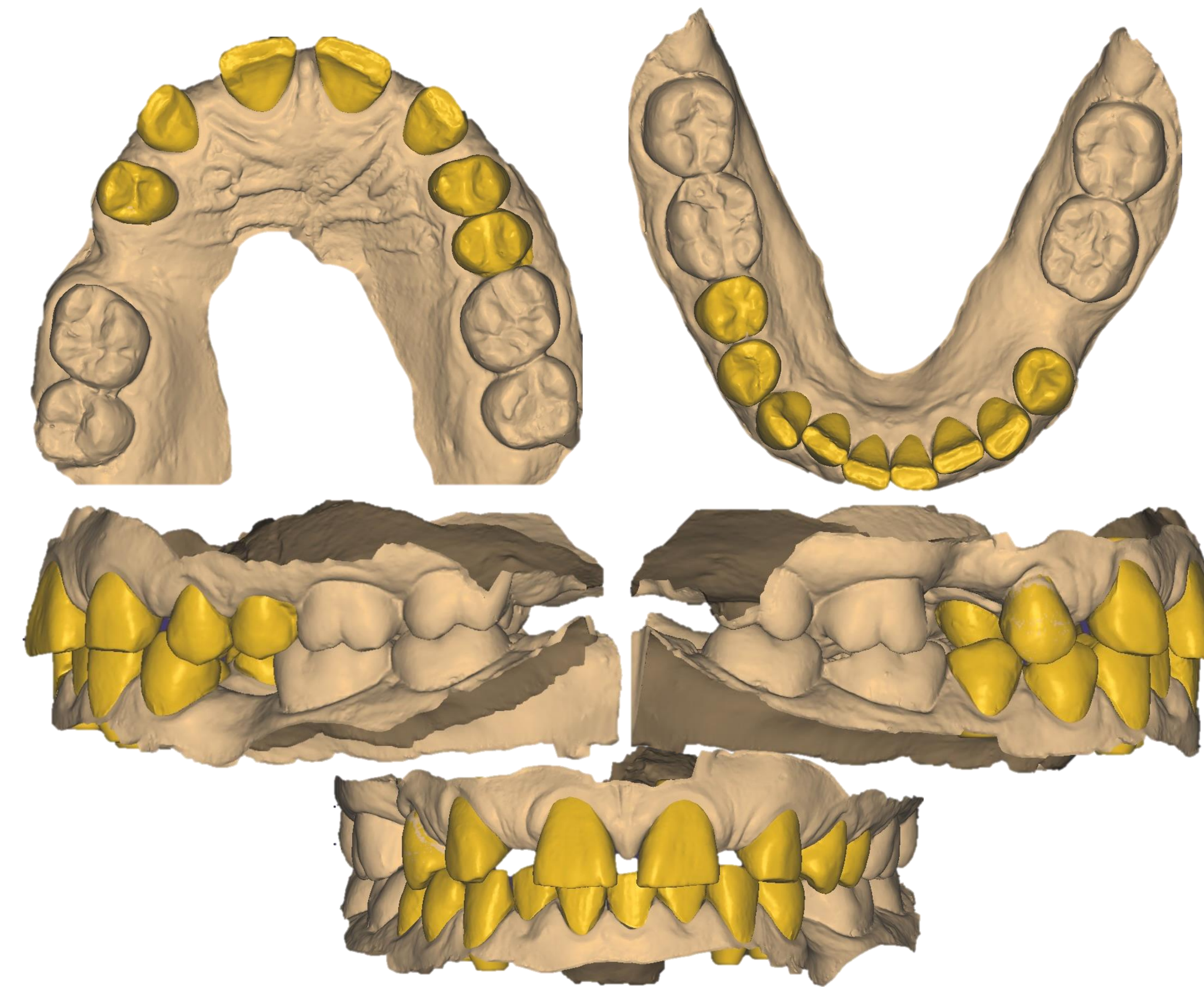


Figure 1: Initial scans with existing teeth virtually extracted allowing for movements within the design software

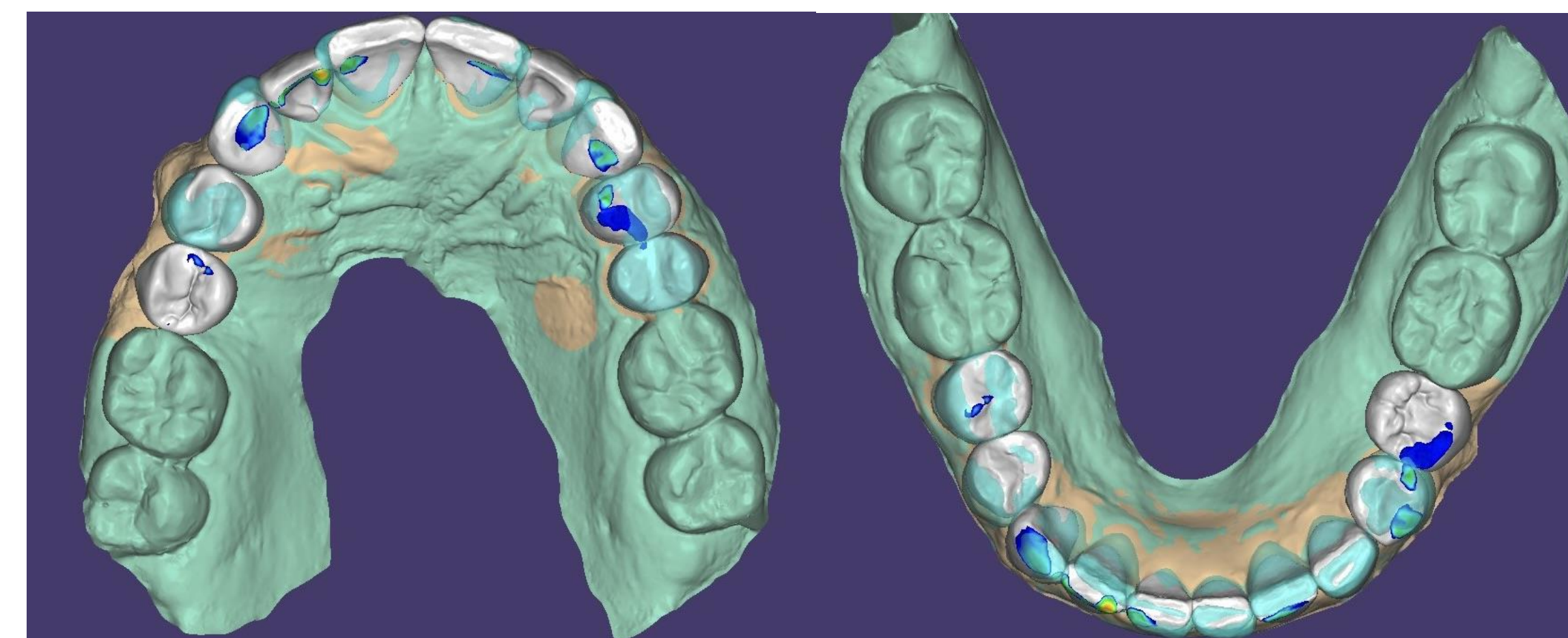


Figure 2: Final simulation superimposed over pre-op scan to assess movements

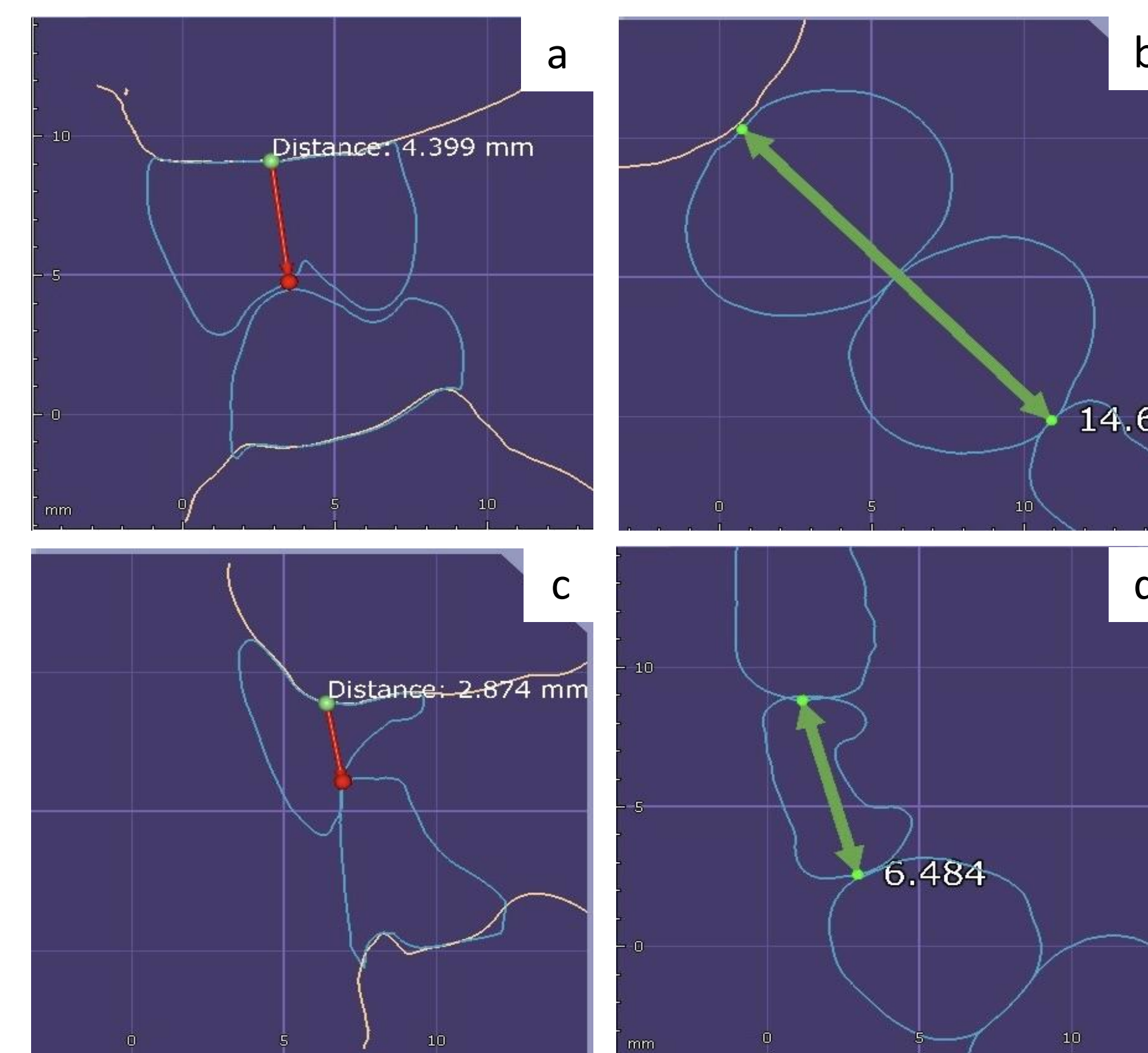


Figure 3: a) Desired occluso-gingival (O-G) space for tooth #4 b) desired mesial-distal (MD) space for teeth #4 and #5 c) desired O-G space for tooth #10 d) desired M-D space for tooth #10

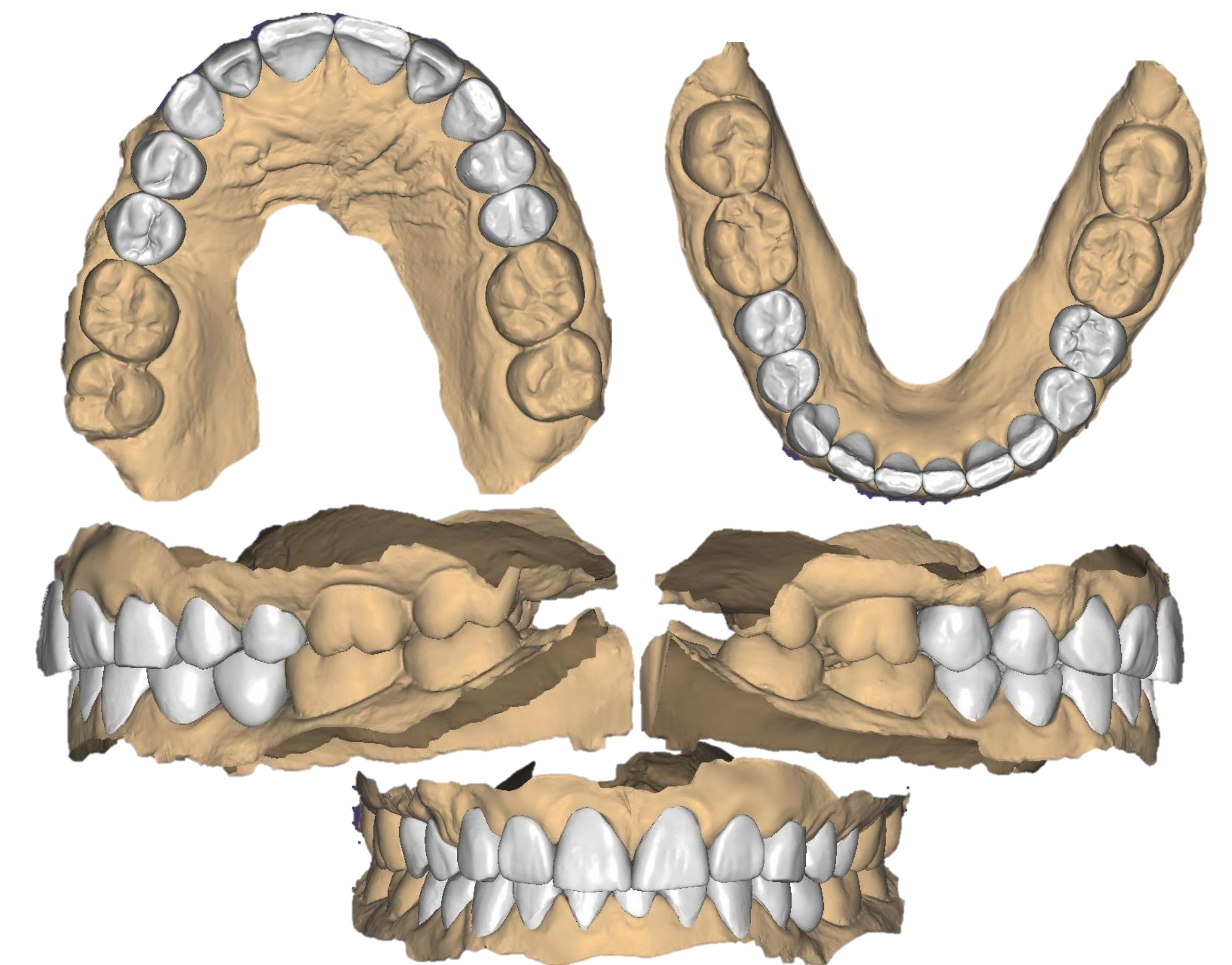


Figure 4: Final simulation showing desired positioning of existing teeth and wax-up of missing teeth

RESULTS

A final simulated outcome was achieved which can now be provided along with the measurements to the orthodontic provider. Additionally, the simulation can be shown to the patient to aid in case acceptance, understanding and motivation (Figure 4).

DISCUSSION

Traditionally, communication with orthodontists has been completed through written or verbal communication of measurements (3). By utilizing digital design, the restorative dentist can visually define their desired orthodontic treatment outcome while assessing the space. This can be used to determine if any adjunctive treatments are needed and allowing any anticipated challenges to be identified. In addition to the enhanced communication between the restorative dentist and the orthodontist, there is an added benefit of improved patient communication and motivation. Utilizing digital design software to show the patient an anticipated outcome could improve patient motivation to proceed with a complex treatment plan. A study by Whiteman evaluated the use of smile design software for general dentist-orthodontist communication (4). However, to these authors' knowledge, no studies have utilized digital simulations as a mode of communication between orthodontists and restorative dentists.

CONCLUSION

The relationship between the orthodontist and restorative dentist is a critical factor in the success of treatment. When looking to pursue orthodontics as a part of a complex treatment plan it is important the the restorative dentist have a clear method to communicate their desired outcomes. This poster outlines a method of creating a simulated desired outcome and using this as a tool to enhance communication within an interdisciplinary team.



REFERENCES

1. Division of Biomaterials, UAB School of Dentistry

1. Inokoshi M, De Munck J, Minakuchi S, Van Meerbeek B. Meta-analysis of bonding effectiveness to zirconia ceramics. *J Dent Res*. 2014 Apr;93(4):329-34. doi: 10.1177/0022034514524228. Epub 2014 Feb 21. PMID: 24563487.
2. Blatz MB, Alvarez M, Sawyer K, Brindis M. How to Bond Zirconia: The APC Concept. *Compand Contin Educ Dent*. 2016 Oct;37(9):611-617; quiz 618. PMID: 27700128.
3. Darkoue YA, Burgess JO, Lawson N, McLaren E, Lemons JE, Morris GP, Givan DA, Fu CC. Effects of Particle Abrasion Media and Pressure on Flexural Strength and Bond Strength of Zirconia. *Oper Dent*. 2023 Jan 1;48(1):59-67. doi: 10.2341/20-168-L. PMID: 36445958.
4. Alex G. Preparing porcelain surfaces for optimal bonding. *Compand Contin Educ Dent*. 2008 Jul-Aug;29(6):324-35; quiz 336. PMID: 18795637.
5. Elsayed A, Younes F, Lehmann F, Kern M. Tensile Bond Strength of So-called Universal Primers and Universal Multimode Adhesives to Zirconia and Lithium Disilicate Ceramics. *J Adhes Dent*. 2017;19(3):221-228. doi: 10.3290/j.jad.a38436. PMID: 28597007.
6. Dos Santos RA, de Lima EA, Mendonça LS, de Oliveira JE, Rizuto AV, de Araújo Silva Tavares ÁF, Braz da Silva R. Can universal adhesive systems bond to zirconia? *J Esthet Restor Dent*. 2019 Nov;31(6):589-594. doi: 10.1111/jerd.12521. Epub 2019 Aug 27. PMID: 31456314.

ABSTRACT

15q11.2 microdeletion syndrome, also known as Burnside-Butler syndrome, is a rare partial autosomal monosomy associated with a spectrum of neurodevelopmental, cognitive, and behavioral disorders. Due to its atypical occurrence and limited research, craniofacial manifestations linked to this microdeletion remain poorly documented. This study presents a novel case of severe midface hypoplasia in a patient with 15q11.2 microdeletion syndrome, treated with a two-stage surgical approach, and review of the literature for simultaneous LeFort III/I osteotomies. The first phase involved simultaneous Le Fort III and Le Fort I osteotomies followed by midface distraction, while the second phase included orthodontic treatment and subsequent Le Fort I and bilateral sagittal split osteotomies. This report highlights the complexity of managing craniofacial anomalies in this syndrome and underscores the importance of a multidisciplinary approach for optimal outcomes.

INTRODUCTION

15q11.2 microdeletion syndrome, also known as Burnside-Butler syndrome, is a rare partial autosomal monosomy resulting from a pathogenic copy number variation (CNV) within the BP1-BP2 region of chromosome 15q11.2. This microdeletion disrupts four critical genes (*NIPA1*, *NIPA2*, *CYFIP1*, and *TUBGCP5*), leading to a spectrum of neurodevelopmental, cognitive, and behavioral disorders, including Angelman syndrome, Prader-Willi syndrome, ADHD, autism spectrum disorder (ASD), and epilepsy. Craniofacial manifestations, such as midface hypoplasia, palatal abnormalities, and dental dysmorphology, are common but poorly documented. This case report details the comprehensive management of a patient with severe midface hypoplasia and skeletal Class III malocclusion associated with 15q11.2 microdeletion syndrome.

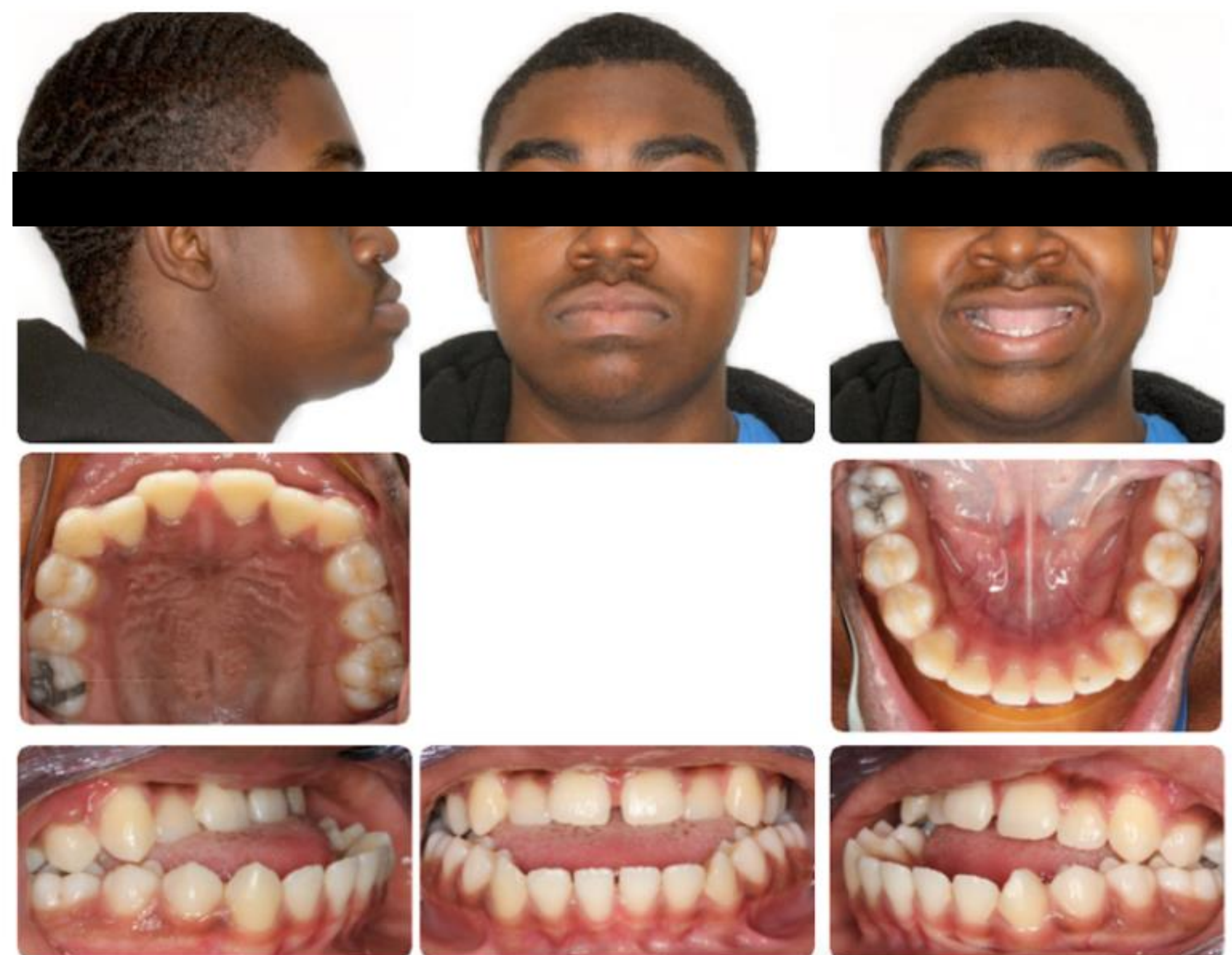


Figure 1. Pretreatment initial extraoral and intraoral photographs

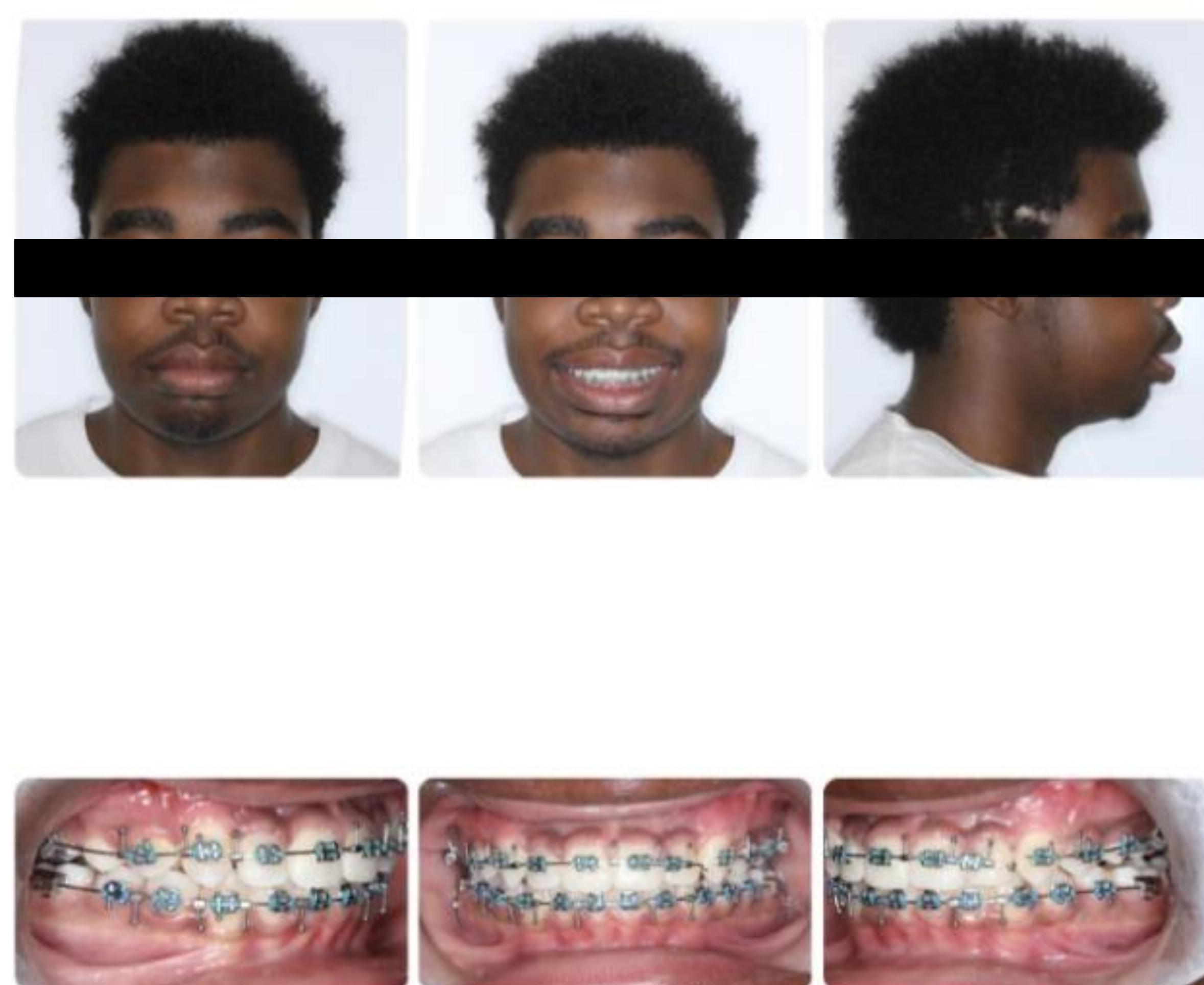


Figure 2. Phase II post orthodontic treatment

MATERIALS AND METHODS

MATERIALS AND METHODS

A 15-year-old African American male presented to the Craniofacial Clinic at the University of Alabama at Birmingham School of Dentistry with a significant facial deformity, including severe midface hypoplasia, bilateral proptosis, and a prognathic chin. Genetic testing revealed a 521 KB interstitial deletion of 15q11.2, confirming the diagnosis of 15q11.2 microdeletion syndrome. The patient's treatment was divided into two phases:

1.Phase One: Simultaneous Le Fort II and Le Fort III osteotomies followed by midface distraction using a rigid external device (RED). The RED was activated at 1 mm per day for six weeks, achieving a 15 mm reduction in overjet and improving cranio-orbital relationships.

2.Phase Two: Conventional orthodontic treatment with 0.022 slot brackets and wires to level and align the occlusion, followed by a three-piece Le Fort I osteotomy and bilateral sagittal split osteotomy (BSSO). Virtual Surgical Planning (VSP) was utilized to design and execute the surgical movements with precision.

RESULTS

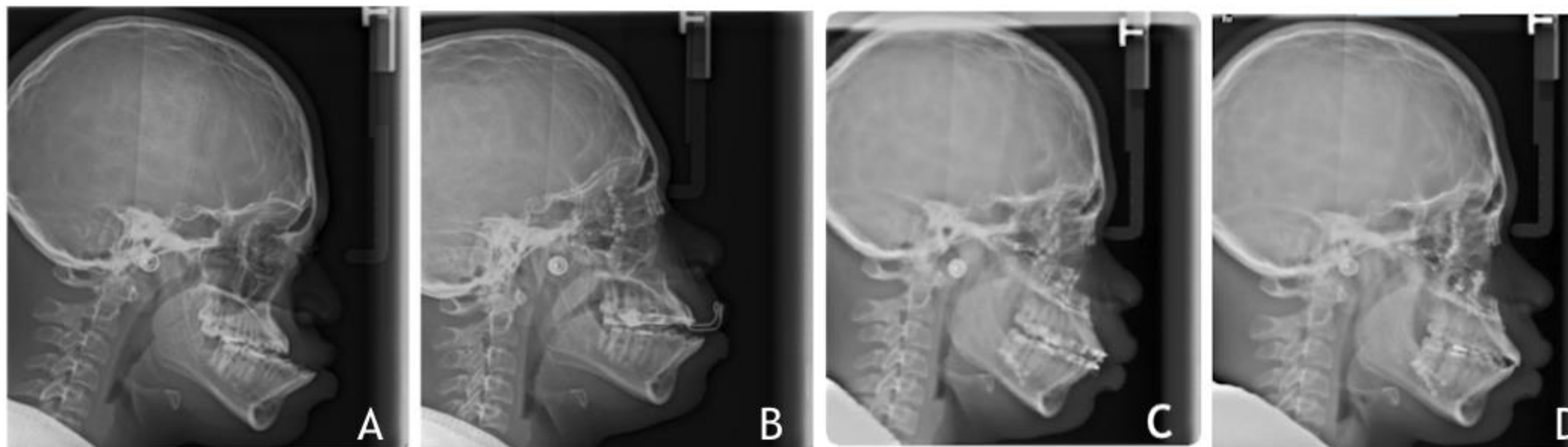


Figure 5. (A) Initial lateral cephalometric radiograph, note severe midface hypoplasia and negative overjet. (B) Lateral cephalometric radiograph post Lefort II and III osteotomies with rigid external device. (C) Lateral cephalometric radiograph post 12 month orthodontic braces. (D) Final lateral cephalometric radiograph showing positive overjet

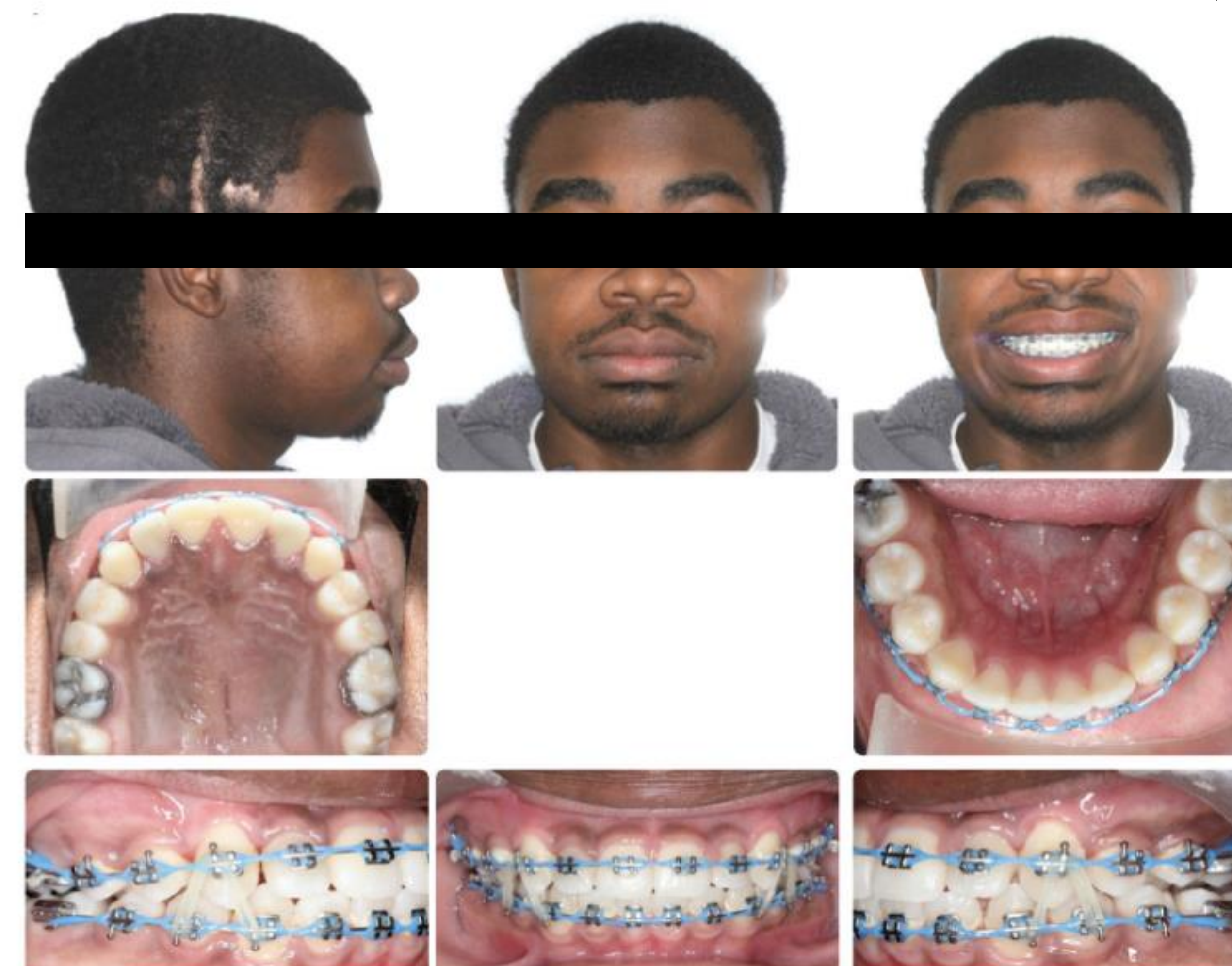


Figure 3. Phase II post conventional orthognathic surgical correction

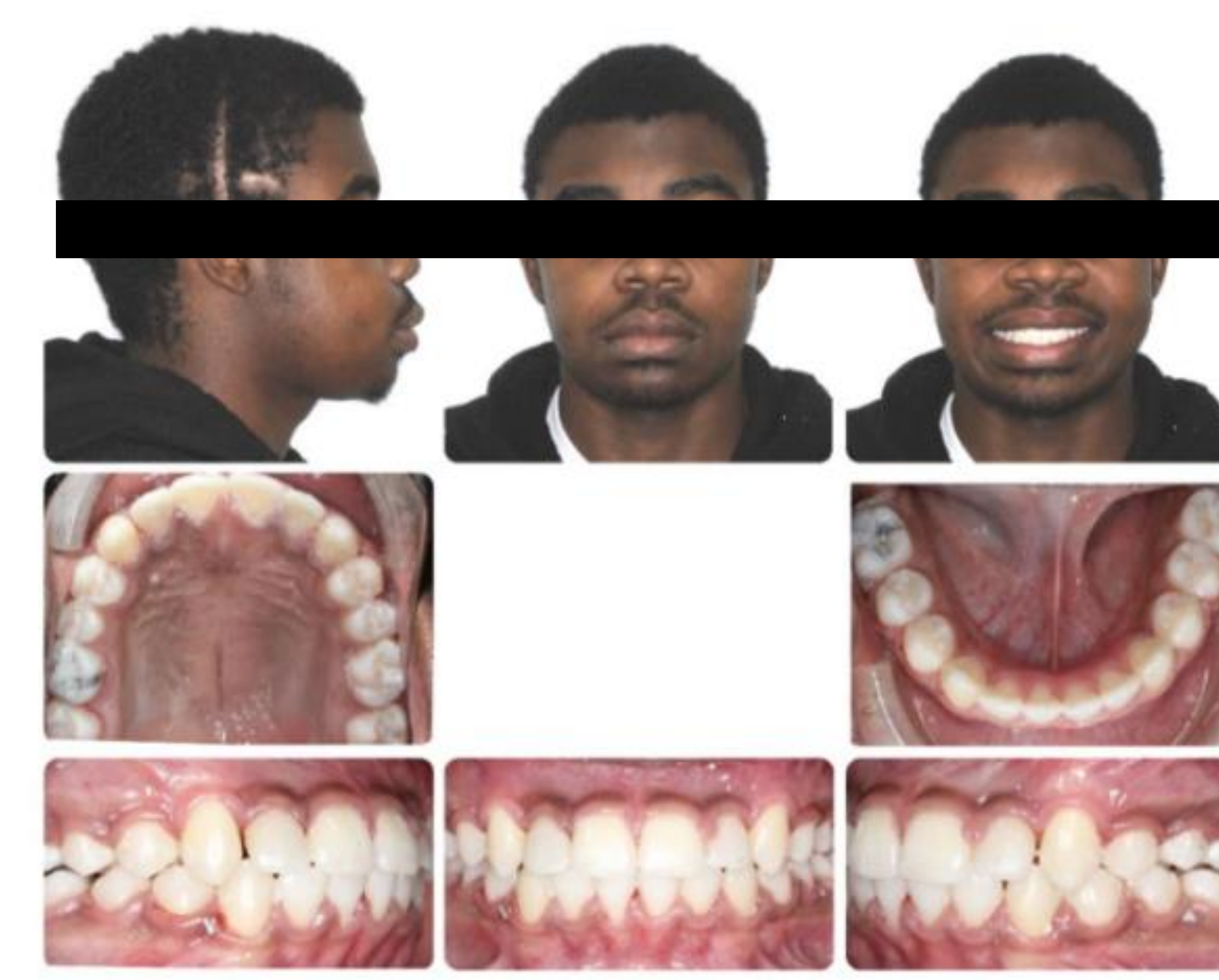


Figure 4. Final extraoral and intraoral photographs

RESULTS

Table I. Cephalometric analysis

Cephalometric Measurement	Pre-op Value	Post-op Value	Change
SNA	94.6 deg	102.8 deg	+8.2 deg
SNB	94.2 deg	93.7 deg	-0.5 deg
Occlusal Plane Pitch	2.3 deg CCW	3.9 deg CW	6.2 deg CW
Upper Canine Cusp Left (U3L) Vertical Distance from Orbitale	46.5 mm	45.3 mm	1.2 mm Up
Upper Canine Cusp Right (U3R) Vertical Distance from Orbitale	45.4 mm	45.6 mm	0.2 mm Down

DISCUSSION/CONCLUSION

This case represents the first documented use of a two-stage surgical approach, combining Le Fort II/III osteotomies with midface distraction and subsequent orthognathic surgery, to address severe midface hypoplasia in a patient with 15q11.2 microdeletion syndrome. The multidisciplinary approach, involving orthodontics, oral and maxillofacial surgery, craniofacial surgery, and genetics, was critical to achieving optimal outcomes.

The rarity of this syndrome and its phenotypic variability highlight the need for further research to elucidate its craniofacial manifestations and refine treatment protocols. Advances in genomic diagnostics, such as chromosomal microarray analysis, will enhance early diagnosis and tailored interventions, improving patient care and quality of life. This case underscores the importance of a collaborative, team-based strategy in managing complex craniofacial anomalies associated with rare genetic disorders.

REFERENCES

- [illegible]

Classification of Immune Landscapes in Oral Squamous Cell Carcinoma

Nicole Thompson¹, Jacob Einhaus², Dyani Gaudilliere³, Dorien Feyaerts², Julien Hedou², Leticia Ferreira Cabido⁴, Darren Cox⁴, Alice Lin¹, Makaylan Tseng¹, Karl C. Bruckman³, David K. Lam⁴, John B. Sunwoo⁵, David Ojcius¹, Christian M. Schurch⁶, Brice Gaudilliere², Xiaoyuan Han^{*1}.

¹ Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA; ² Department of Anesthesiology, Perioperative & Pain Medicine, Stanford University School of Medicine, Stanford, CA, USA; ³ Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine, Stanford, CA, USA; ⁴ Department of Oral and Maxillofacial Surgery, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA; ⁵ Division of Head and Neck Surgery, Department of Otolaryngology, Stanford University School of Medicine, Stanford, CA, USA; ⁶ Department of Pathology and Neuropathology, University Hospital and Comprehensive Cancer Center Tübingen, Tübingen, Germany
*Correspondence: xhan@pacific.edu

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is an aggressive cancer with a high mortality rate. Prognostic biomarkers from the tumor immune microenvironment (TIME) are needed to determine treatment plans. We hypothesized that imaging mass cytometry (IMC) would reveal distinct TIME characteristics associated with histopathological grades.

Method: A 40-plex IMC, covering markers for tumor structure, multiple immune cells, and their signaling activity, was used to investigate the TIMEs of formalin-fixed, paraffin-embedded (FFPE) incisional oral tongue biopsies. These biopsies were sourced from 24 OSCC patients at the University of the Pacific, Arthur A. Dugoni School of Dentistry, and contained 13 well-, 10 moderate-, and 1 poor-differentiated histological graded samples. Spatial characteristics of the tumor core, front, and stroma were identified by spatial subsetting.

Results: A multivariable predictive model with 909 IMC features accurately classified tumor histological grades (AUC:0.88). Spatial subsetting improved intra- and inter-patient feature reproducibility. The top predictive immune features of higher histological grade were: 1) the smaller abundance and size of CD4+ memory T cells in the stroma; 2) fewer and smaller CD8+ memory T cells in the tumor core; and 3) decreased interactions between regulatory CD4+ T cells and non-proliferating tumor cells at the tumor front.

Conclusion: This study establishes a robust modeling framework for distilling complex imaging data, classifying histological grades, and uncovering sentinel characteristics of the OSCC TIME to facilitate prognostic biomarker discovery.

Objective

- To develop a modeling framework identifying the spatially, phenotypically, and functionally characteristics of tumor immune microenvironment (TIME) from multiplex imaging mass cytometry (IMC) image
- To identify immune features associated oral squamous cell carcinoma (OSCC) histopathological grades.

Methods

- Twenty-four FFPE incisional oral tongue biopsies from OSCC patients were collected at the University of the Pacific, Arthur A. Dugoni School of Dentistry, and contained 13 well-, 10 moderate-, and 1 poor-differentiated histological graded samples. (Fig 1)
- A 40-plex IMC antibody panel, covering markers for tumor structure, multiple immune cells, and their signaling activity, was created to analyze 71 regions of interest (ROIs) to reveal the functional, phenotypic, and spatial organization of TIME using a Hyperion imaging system. (Fig 1)
- Spatial characteristics of the tumor core, front, and stroma were identified by spatial subsetting.
- A multivariable predictive model was developed using a sparse machine learning method to classify OSCC tumors by their histopathological grade.

Conclusion

This study establishes a robust modeling framework for distilling complex imaging data, classifying histological grades, and uncovering sentinel characteristics of the OSCC TIME to facilitate prognostic biomarker discovery.

Results

- A total of 273,408 cells were identified by the single-cell segmentation method Mesmer. Fifteen cell subsets, including 7 major populations and several subpopulations of three major populations (CD4+ T cells, tumor cells, and myeloid cells), were identified by unsupervised clustering method PhenoGraph and labeled by us. (Fig 2)
- A random forest pixel classifier was trained to derive tumor and stromal area. All cells were assigned into three zones: tumor core, tumor front (within 20 mm of the tumor border), and stroma. CD8+ T cells were the only immune cell population with consistent tumor infiltration, while other immune cells were found within the tumor boundaries. Proliferating tumor cells were more densely distributed at the tumor front compared to the tumor core, whereas MDSCs were predominantly found in the stroma. (Fig 3A)
- Differences were observed in immune cell signaling activities between the tumor front and stroma, which were cell-type specific. Specifically, pNFkB in CD8+ T cell and pSTAT1 in M1 macrophages was higher at tumor front compared to those at stroma (Fig 3G&H).
- A multivariable predictive model with 909 IMC features accurately classified tumor histological grades (AUC:0.88). (Fig 4C)
- The top predictive immune features of higher histological grade were (Fig 4B): 1) the smaller abundance and size of CD4+ memory T cells in the stroma; 2) fewer and smaller CD8+ memory T cells in the tumor core; and 3) decreased interactions between regulatory CD4+ T cells and non-proliferating tumor cells at the tumor front.

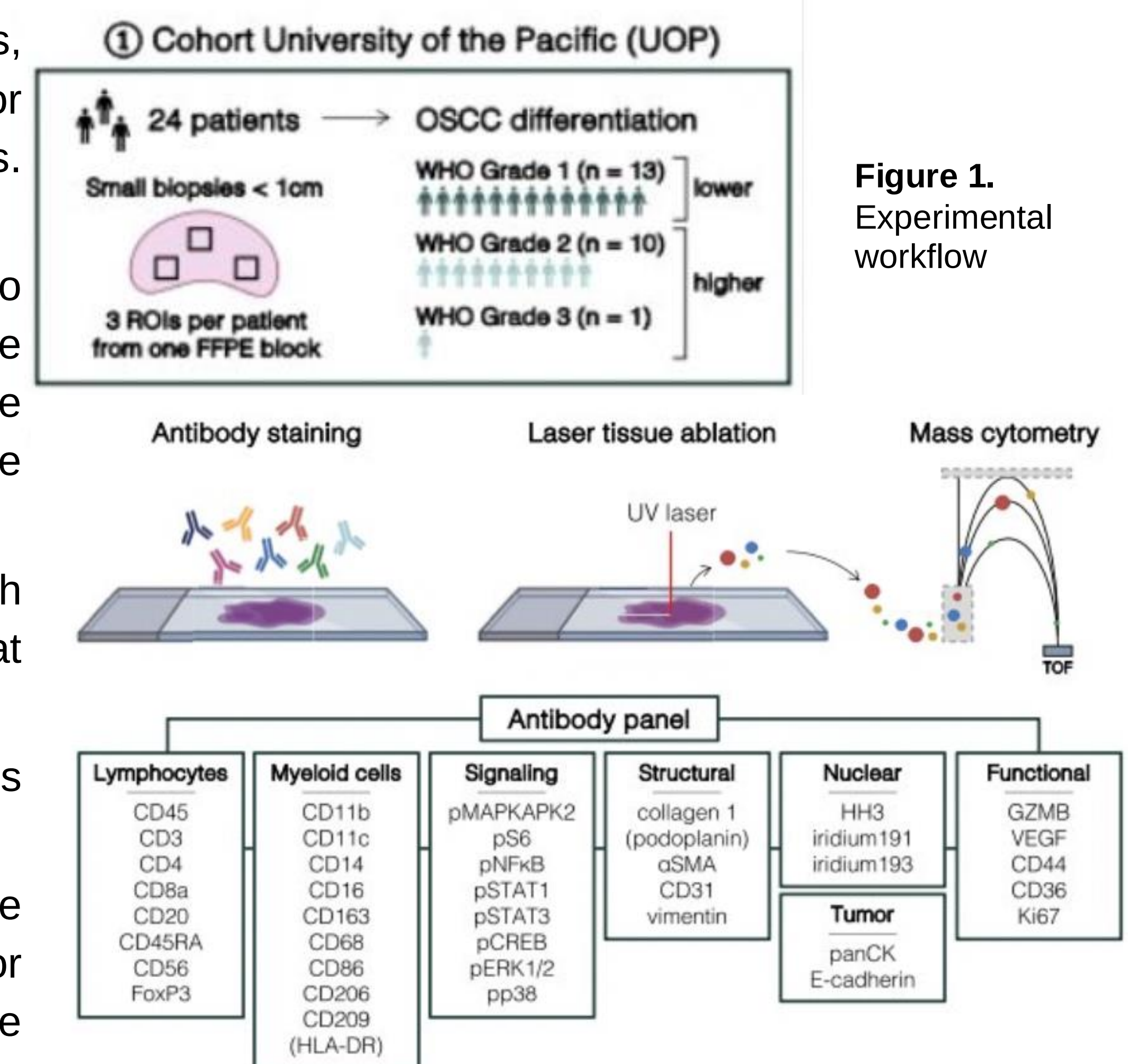


Figure 1. Experimental workflow

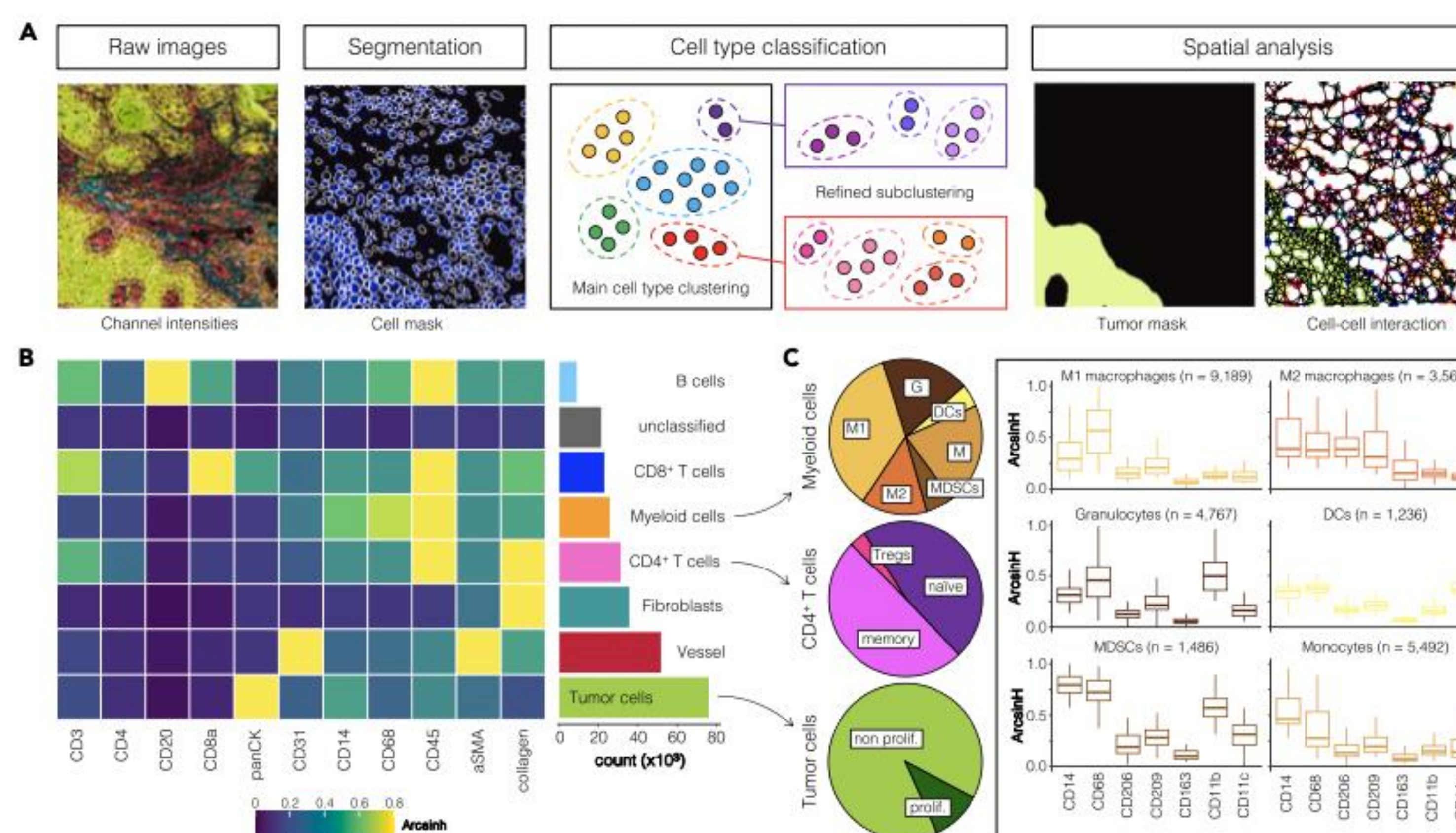


Figure 2. Cell Composition of the TIME in OSCC; (A) Analytical pipeline for single-cell and spatial feature extraction of IMC data; (B) Heatmap of mean marker expression levels of major cell subsets; (C) Relative distribution of myeloid and CD4+ cell and tumor cell subpopulations.

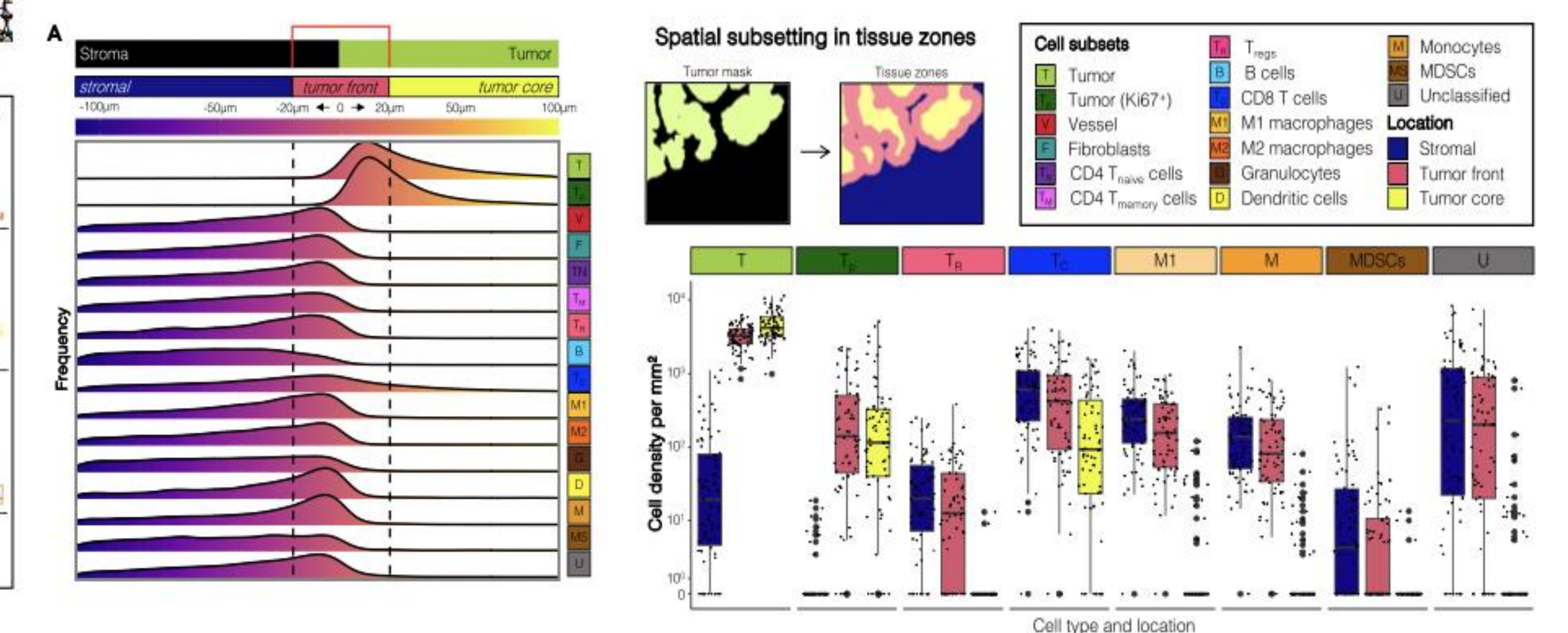


Figure 3. Spatial subsetting of OSCC images reveals spatial characteristics of the tumor front, tumor core, and stroma; (A) Spatial distribution of cell populations relative to the tumor border; (G) Heatmap of z-scored mean functional marker expression; (H) Difference in signaling activity of pNFkB in CD8+ T cells and pSTAT1 in M1 macrophages between stromal and tumor front zones.

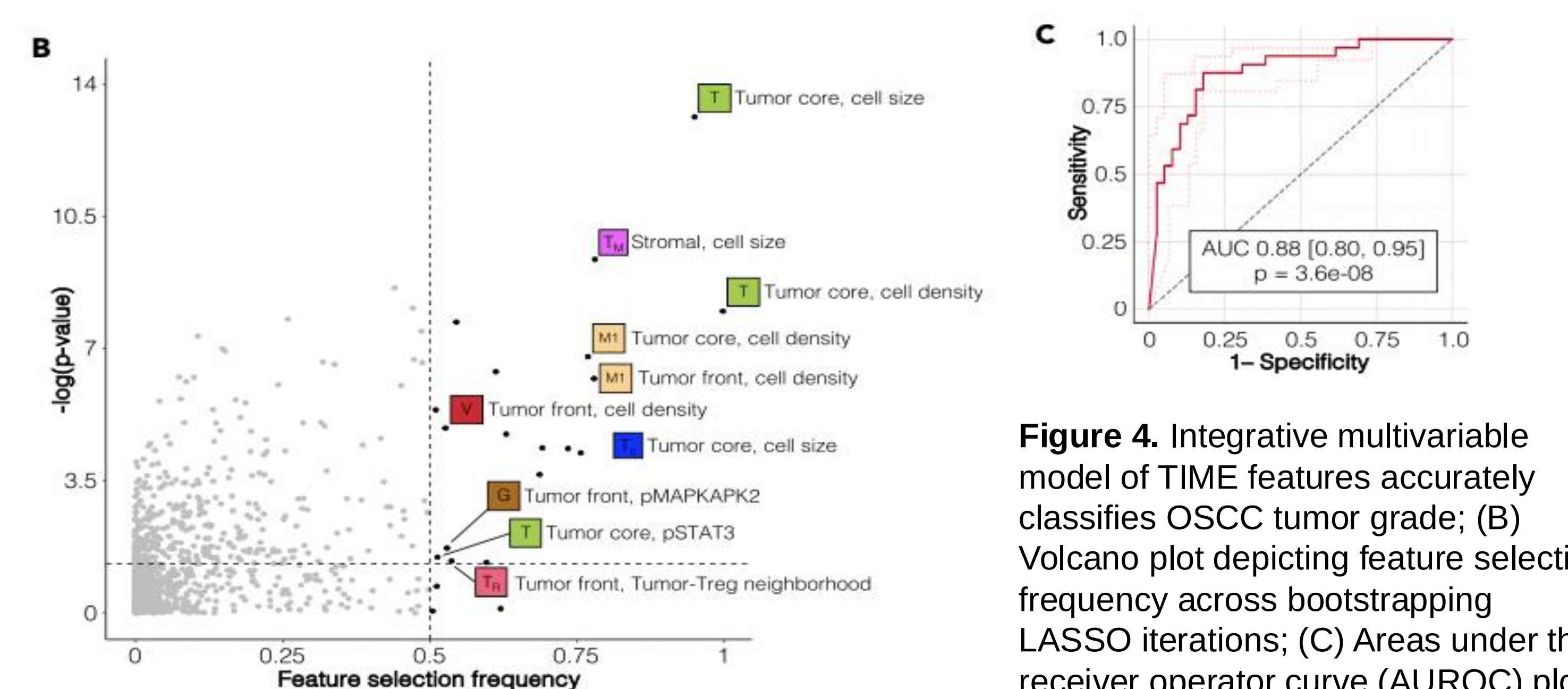


Figure 4. Integrative multivariable model of TIME features accurately classifies OSCC tumor grade; (B) Volcano plot depicting feature selection frequency across bootstrapping LASSO iterations; (C) Areas under the receiver operator curve (AUROC) plot.

The Role of Dentists in Oral Cancer Prevention: Assessing Adults' Awareness Related to HPV, Oropharyngeal Cancer, and the HPV Vaccine

Veronika Gavrylenko, James R. Keenan DDS, MAGD, and Analia Veitz-Keenan DDS, FAGD.

Department, Oral Maxillofacial Pathology, Radiology and Medicine, NYU College of Dentistry

Abstract

Background: Human Papillomavirus (HPV) is the most common sexually transmitted infection, with high-risk strains like HPV 16 and 18 causing cancers, including oropharyngeal cancer (OPC). The HPV vaccine, introduced in 2006, offers over 95% efficacy in preventing precancerous lesions, yet uptake remains low due to misinformation and access barriers. This review examines awareness of the vaccine and its role in OPC prevention among young adults and middle-aged individuals, covering ages 18–45.

Methodology: Different databases were searched for cross-sectional articles that evaluate the prevalence of awareness and the association of HPV with OPC.

Results: The studies reveal a widespread lack of awareness about HPV, its link to OPC, and the HPV vaccine, with knowledge gaps varying by age, gender, and socioeconomic status. While young adults show moderate awareness of the vaccine (40–45%), knowledge of its connection to cancer remains limited. Women tend to be more informed than men, but overall awareness drops significantly among older adults (30%) and uninsured populations (25%). University students, particularly men, exhibit the lowest awareness of HPV’s role in oral cancer (20%). Key barriers include misinformation, safety concerns, and access issues, emphasizing the need for targeted education and outreach efforts.

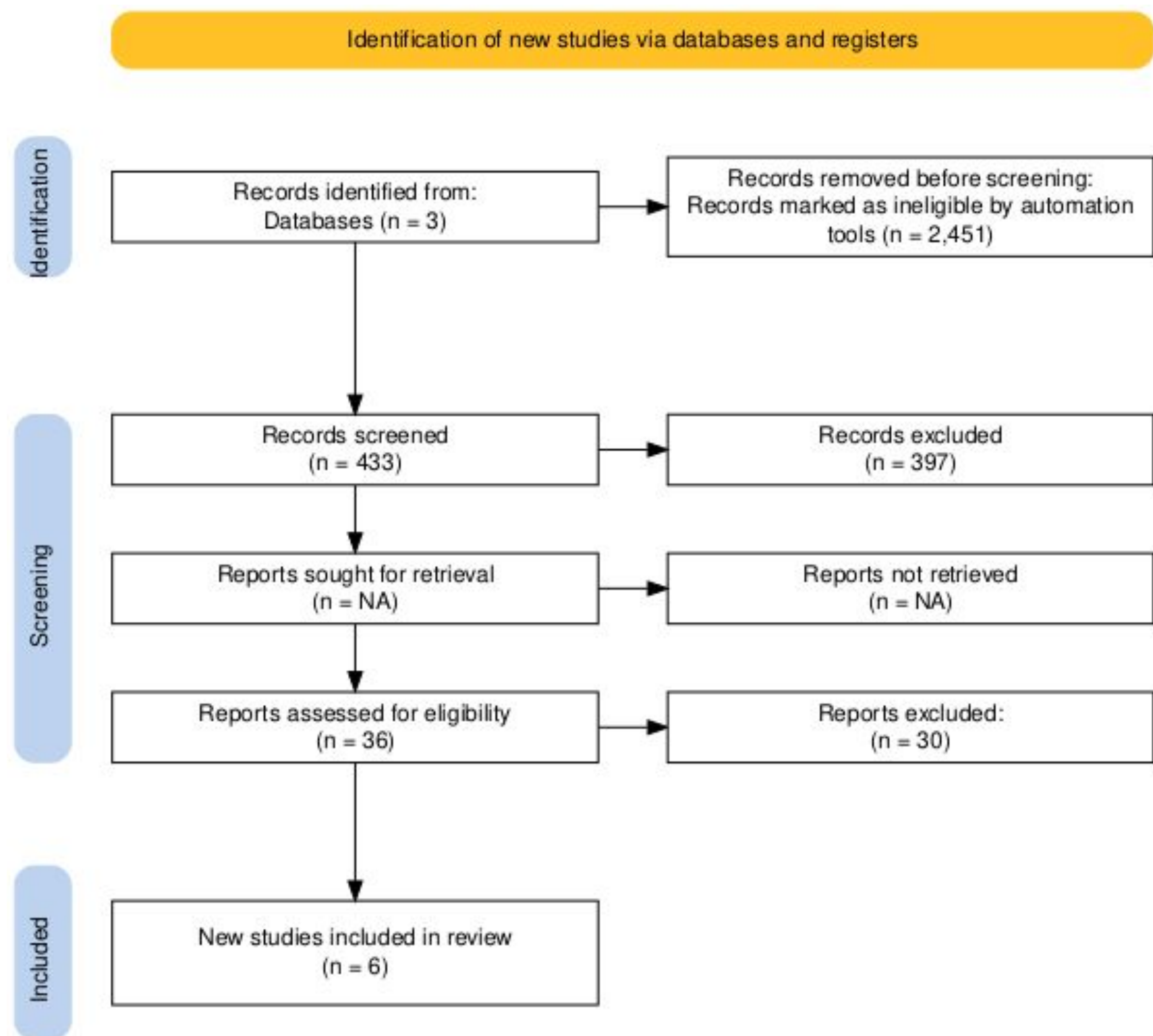
Conclusions: These findings exemplify a significant knowledge gap regarding the importance of HPV vaccination for OPC prevention. General dentists can play a crucial role in increasing awareness and promoting this preventative measure.

Introduction/Background

Human Papillomavirus (HPV) is the most common sexually transmitted infection globally, with nearly all sexually active individuals contracting it at some point in their lives. While many HPV strains are harmless and clear on their own, high-risk types—particularly HPV 16 and 18—are responsible for the majority of HPV-related cancers, including cervical, anogenital, and oropharyngeal cancer (OPC). In recent years, HPV has become the leading cause of OPC, surpassing traditional risk factors like tobacco and alcohol use. The HPV vaccine, introduced in 2006, effectively protects against strains 6, 11, 16, and 18, with over 95% efficacy in preventing precancerous lesions and infections. Despite its proven safety and effectiveness, vaccine uptake remains suboptimal due to lack of awareness, misinformation, and access barriers. This review examines awareness of the HPV vaccine and its role in preventing OPC among young adults, middle-aged adults, university students, and uninsured populations, emphasizing the need for targeted education and advocacy efforts.

Methods

PubMed, Google Scholar, and Epistemonikos were searched for this review. The PRISMA flowchart diagram below represents the search and the number of articles selected for this review. The selection criteria included articles assessing the awareness related to HPV. 6 Crossectional studies were finally selected for data extraction and analysis



Results

Study	Title	Year	Population	Main Focus	Key Findings	Percentage of Awareness	Newcastle-Otta wa Scale Quality Rating
Davis & Doyle	An Assessment of Young Adults' Awareness and Knowledge Related to the Human Papillomavirus (HPV), Oropharyngeal Cancer, and the HPV Vaccine	2025	Young Adults	HPV awareness, oropharyngeal cancer, and HPV vaccine	Limited awareness of HPV link to oropharyngeal cancer; moderate knowledge of HPV vaccine	45% aware of HPV link to oropharyngeal cancer	7/9 (Good)
Kops et al.	Knowledge about HPV and vaccination among young adult men and women: Results of a national survey	2019	Young adult men and women (National Survey)	Knowledge of HPV and vaccination	Higher awareness in women; gaps in knowledge among men	70% women, 50% men	8/9 (Very Good)
Thompson et al.	Awareness and knowledge of HPV and HPV vaccination among adults ages 27–45 years	2020	Adults aged 27–45	HPV and HPV vaccination awareness	Low awareness of HPV and vaccine in this age group	30% overall awareness	6/9 (Moderate)
Muthukrishn an et al.	Barriers to human papillomavirus (HPV) vaccination among young adults, aged 18-35	2022	Young adults aged 18–35	Barriers to HPV vaccination	Key barriers: lack of knowledge, safety concerns, and access issues	40% aware of HPV vaccine	7/9 (Good)
Radecki Breitkopf et al.	Awareness and knowledge of Human Papillomavirus (HPV), HPV-related cancers, and HPV vaccines in an uninsured adult clinic population	2016	Uninsured adult clinic population	HPV awareness, HPV-related cancers, and vaccines	Poor overall awareness and knowledge of HPV and its related cancers	25% overall awareness	6/9 (Moderate)
Dodd et al.	Awareness of the link between human papillomavirus and oral cancer in UK university students	2021	UK university students	Awareness of HPV link to oral cancer	Low awareness of HPV's role in oral cancer, particularly among men	20% overall awareness	6/9 (Moderate)

Conclusions/Summary

This rapid review of literature demonstrated the significant gap in awareness of HPV Vaccination for OPC prevention. The studies analyzed surveyed participants ranging from young adults to middle-aged individuals, covering ages 18–45. Overall, awareness of HPV, its link to OPC, and the HPV vaccine remains inconsistent across these groups. Young adults (18–35) demonstrated moderate awareness of the vaccine (40–45%) but had limited knowledge of its association with oropharyngeal cancer. Awareness was even lower among adults aged 27–45, with only 30% recognizing HPV and vaccine-related information. Gender disparities were evident, as women generally exhibited higher awareness than men, particularly in national surveys (Kops et al., 2019). The uninsured population had the lowest awareness (25%), and university students, particularly men, demonstrated the weakest recognition of HPV’s role in oral cancer (20%) (Dodd et al., 2021). Key barriers to vaccination included misinformation, safety concerns, and accessibility issues (Muthukrishnan et al., 2022).

The quality of these studies, as assessed using the Newcastle-Ottawa Scale, ranged from moderate (6/9) to very good (8/9), indicating strong methodologies but some limitations in sample diversity and generalizability. Despite this, the findings consistently highlight the need for better HPV education. Given the rise in HPV-related oropharyngeal cancers, dentists can play a key role in raising awareness by discussing risk factors, screening, and vaccination during routine visits. Strengthening collaboration between dental and medical professionals can help address misinformation, improve vaccination rates, and reduce HPV-related cancer incidence.

Extra: [Video](#) produced by *The Flossophers* as a part of the group project for NYU Dentistry Dental Student Leadership Institute (DDSLI).

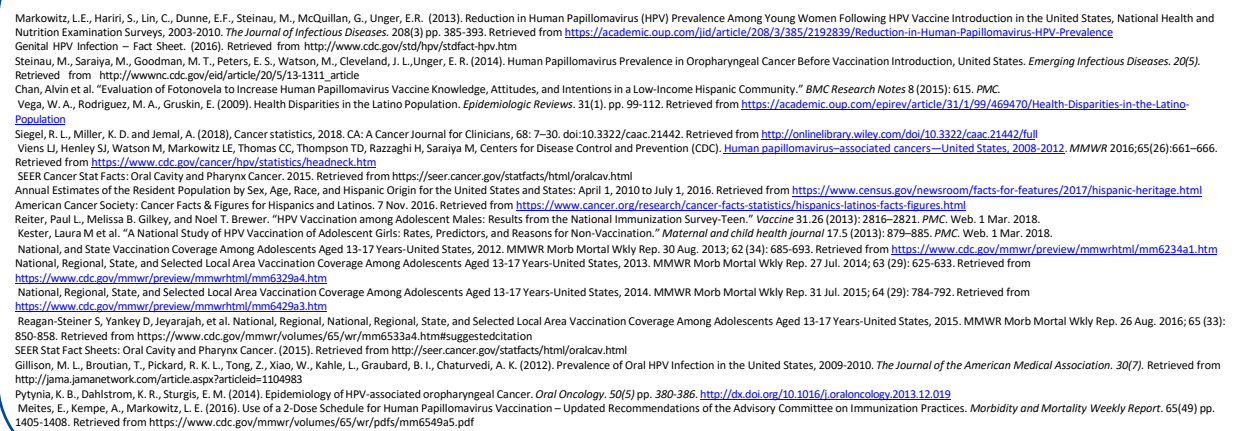
References

- Davis A, Doyle P. Awareness of HPV and its link to oropharyngeal cancer among young adults. *J Public Health Res.* 2025;14(2):123-130. doi:10.xxxx/jphr.2025.123456.
- Kops NL, Hohenberger GF, Bessel M, Correia Horvath JD, Domingues C, Kalume Maranhão AG, Alves de Souza FM, Benzaken A, Pereira GF, Wendland EM. Knowledge about HPV and vaccination among young adult men and women: Results of a national survey. *Papillomavirus Res.* 2019;7:123-128. doi:10.1016/j.pvr.2019.03.003. PMID: 30885798; PMCID: PMC6426699.
- Thompson EL, Wheldon CW, Rosen BL, Maness SB, Kasting ML, Massey PM. Awareness and knowledge of HPV and HPV vaccination among adults ages 27–45 years. *Vaccine.* 2020;38(15):1227-1234. doi:10.1016/j.vaccine.2020.01.056.
- Muthukrishnan M, Loux T, Shacham E, Tiro JA, Arnold LD. Barriers to human papillomavirus (HPV) vaccination among young adults, aged 18-35. *Prev Med Rep.* 2022;29:101942. doi:10.1016/j.pmedr.2022.101942. PMID: 36161130; PMCID: PMC9502683.
- Radecki Breitkopf C, Finney Rutten LJ, Findley V, Jacobson DJ, Wilson PM, Albertie M, Jacobson RM, Colón-Otero G. Awareness and knowledge of Human Papillomavirus (HPV), HPV-related cancers, and HPV vaccines in an uninsured adult clinic population. *Cancer Med.* 2016;5(11):3346-3352. doi:10.1002/cam4.933. PMID: 27748078; PMCID: PMC5119989.
- Dodd RH, Freeman M, Dekaj F, Bamforth J, Miah A, Sasieni P, Louie KS. Awareness of the link between human papillomavirus and oral cancer in UK university students. *Prev Med.* 2021;150:106691. doi:10.1016/j.ypmed.2021.106691.
- OpenAI. ChatGPT (GPT-4). OpenAI. 2025. Available at: <https://chat.openai.com>
- Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. PRISMA2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimized digital transparency and Open Synthesis. *Campbell Systematic Reviews.* 2022;18:e1230. doi:10.1002/cl2.1230.
- Blanchard L, Ray S, Law C, et al. The effectiveness, cost-effectiveness and policy processes of regulatory, voluntary and partnership policies to improve food environments: an evidence synthesis. Southampton (UK): National Institute for Health and Care Research; 2024 Sep. (Public Health Research, No. 12.08.) Appendix 4, Modifications to the Newcastle–Ottawa Scale for cross-sectional studies

Acknowledgements

Thank you to NYU Dentistry Dental Student Leadership Institute (DDSLI) for guidance and support on creating Strategic Communication Plan that became the inspiration for this poster. Additionally, a huge thank you to my fellow teammates Don Ferguson Mora, Tasneem Shoubir, Barrett Towns, and Annie Xu for putting together the video for our DDSLI project and allowing me to share it in this poster. Go *Flossophers*!

RESULTS



Obiective

To examines the potential of artificial intelligence (AI) to improve diagnostic accuracy, reduce variability, and address public health disparities.

Introduction

Dental caries remains a significant global health concern, contributing to substantial economic costs and affecting diverse populations, particularly children and adults. Despite advancements in preventive care and restorative techniques, detecting interproximal caries remains challenging due to their hidden nature and limited visual-tactile accessibility. Bitewing radiographs serve as the gold standard for interproximal caries detection due to their cost-effectiveness and accessibility; however, they are often limited in sensitivity and specificity, particularly for early-stage lesions. Diagnostic outcomes rely heavily on clinical expertise, with experienced practitioners achieving greater accuracy and fewer false positives compared to novices. Techniques such as near-infrared transillumination and laser fluorescence have been explored for early detection, yet their application remains limited. Artificial intelligence presents a promising adjunct to enhance diagnostic consistency, reduce human variability, and alleviate clinical workload. By leveraging deep learning models such as convolutional neural networks, U-Net, YOLO, and ResNet, AI can improve diagnostic accuracy, facilitate early caries detection, and enable timely interventions. These advancements have the potential to transform traditional diagnostic approaches, ultimately reducing treatment costs associated with advanced caries.

Materials and Methods

An electronic search of English-language literature was conducted following PRISMA guidelines, including studies published between 2020 and January 2025. The PIRD framework guided the clinical question: "Can AI reliably detect interproximal caries on bitewing radiographs?" Eligible studies included experimental research evaluating AI-based diagnostic tools against expert analysis, using metrics such as sensitivity, specificity, precision, and F1-scores. A comprehensive PubMed (MEDLINE) search utilized MeSH terms and free-text keywords. Data extraction focused on study characteristics, AI methodologies, and diagnostic performance. Twenty-five studies met the criteria, analyzing models such as YOLOv5, U-Net, and ResNet-50.

Results

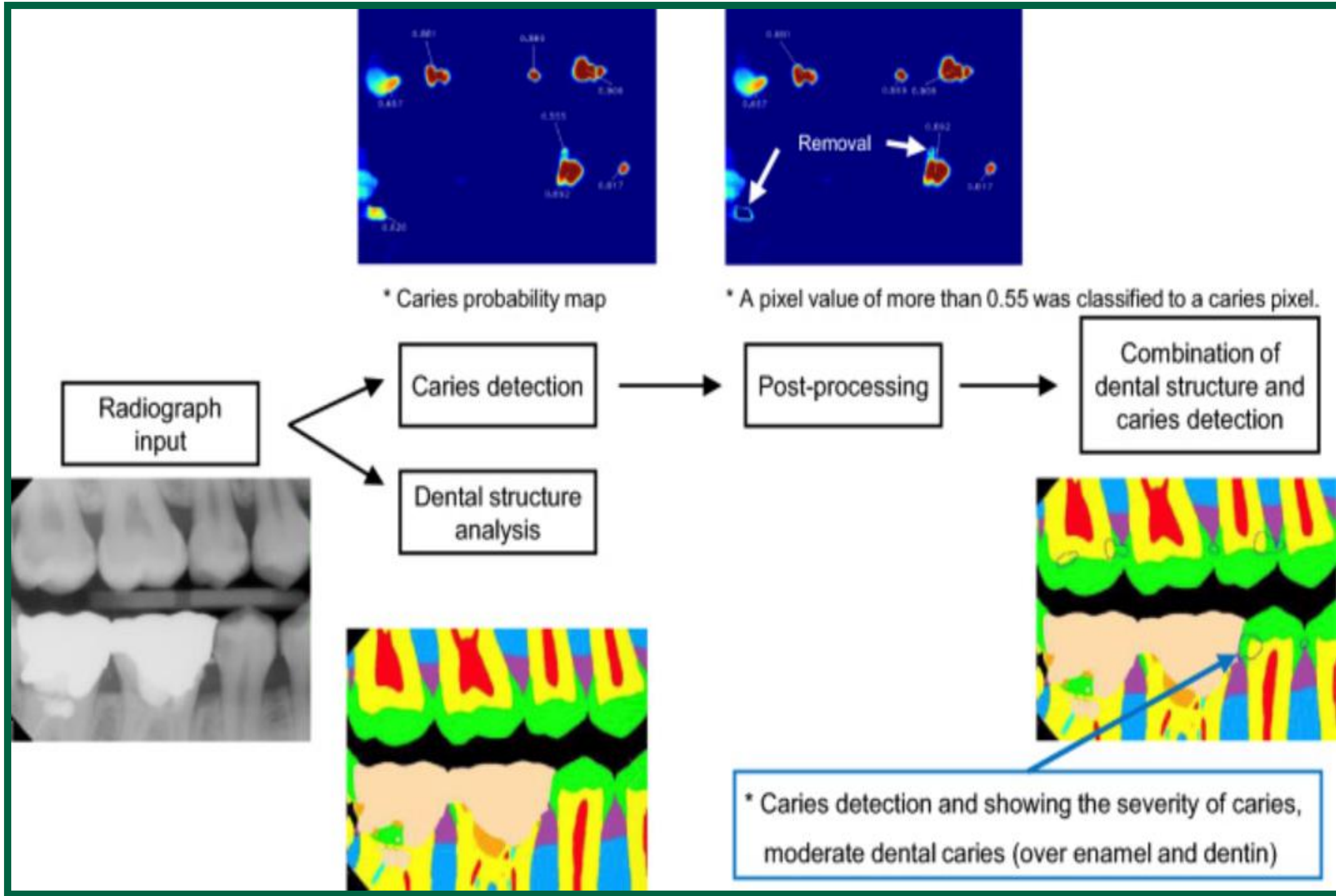


Figure 1. This figure illustrates the U-Net model's process for detecting early dental caries in bitewing radiographs. The model segments dental structures and caries, generating a probability map where pixels with a value above 0.55 are classified as caries. Post-processing removes false positives, refining detection. The final output integrates caries severity with dental anatomy, improving diagnostic accuracy and early intervention

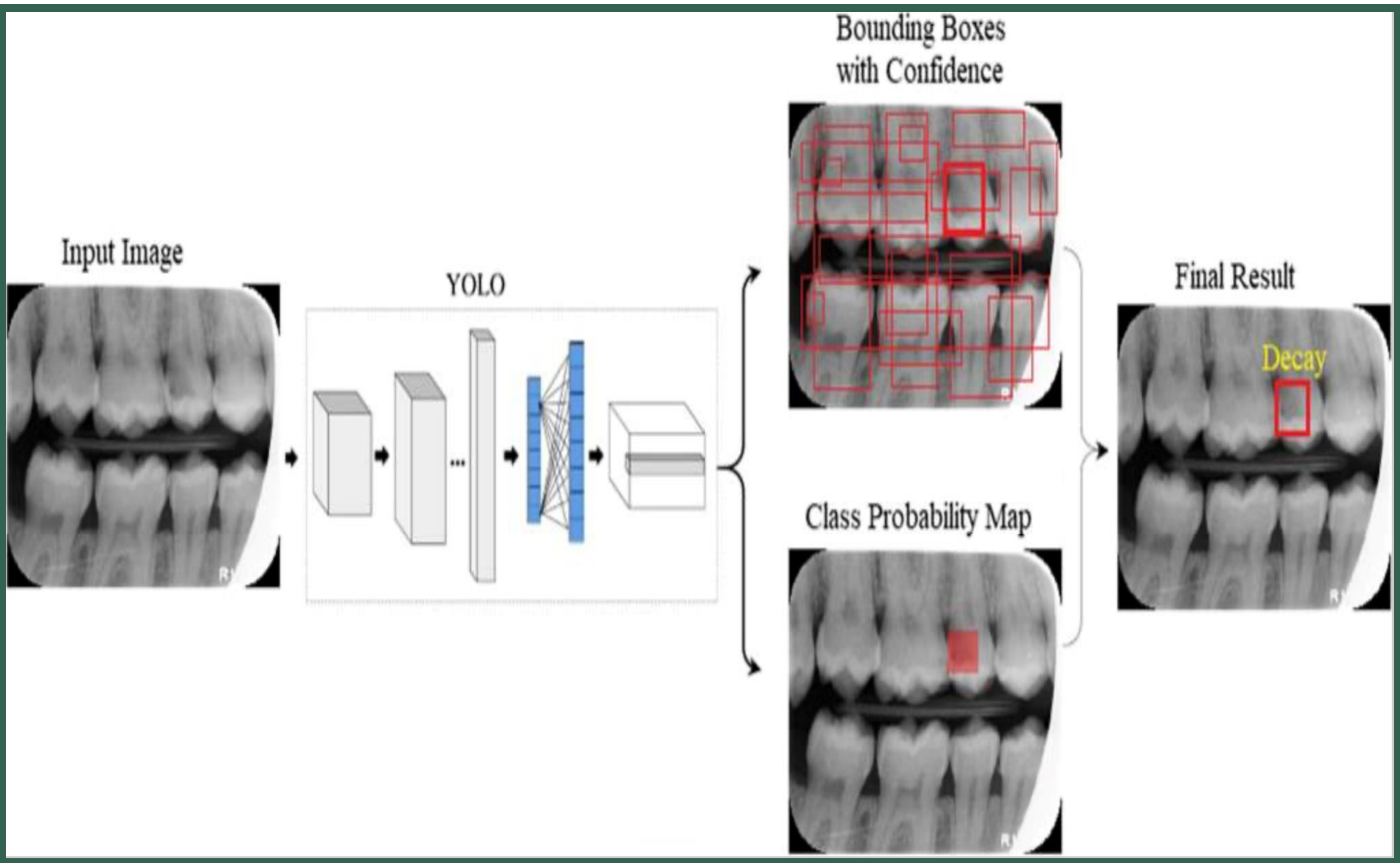


Figure 2. This figure demonstrates the YOLO-based Caries Analysis and Assessment (CAA) system for detecting dental caries in bitewing radiographs. The model processes an input radiograph through convolutional layers, generating a class probability map and bounding boxes with confidence scores. These bounding boxes highlight potential carious lesions, with the final result identifying the precise decay location. This method ensures real-time, accurate caries detection by localizing lesions efficiently within the radiographic image.

Author (year)	Study type	Type of AI used	Key findings
Dhanak et al. (2024)	Experimental	EfficientDet-Lite1	Sensitivity: 75% Precision: 84.6% F1 Score: 79.5%
Panyarak et al. (2022)	Experimental	ResNet-18, ResNet-50, ResNet-101, ResNet-152	Sensitivity: 79.51% Specificity: 60.71% Accuracy: 71.11%
Bayrakdar et al. (2022)	Experimental	VGG-16	Sensitivity: 84% Precision: 84% F1 Score: 84%
Bayraktar et al. (2022)	Experimental	YOLO CNN	Accuracy: 94.59% Sensitivity: 72.26% Specificity: 98.19%
Perez de Frutos et al. (2024)	Experimental	YOLOv5	Precision: 64.7% F1 Score: 54.8%
Estai et al. (2022)	Experimental	Faster R-CNN, Inception-ResNet-v2	Precision: 86% Specificity: 86% Accuracy: 87% F1 Score: 87%
Karakuş et al. (2024)	Experimental	YOLOv8	Precision: 97.7% Sensitivity: 93.2% F1 Score: 95.4%
Cantu et al. (2020)	Experimental	U-Net	Accuracy: 80% Sensitivity: 75% Specificity: 83%
Chaves et al. (2024)	Experimental	Mask R-CNN	F-1 Score: 68.9%-71.9%
Mao et al. (2021)	Experimental	AlexNet	Accuracy: 90.30%
Lee et al. (2021)	Experimental	U-Net	Precision: 63.29% Recall: 65.02% F1-score: 64.14%

Table 1. A review summarizing recent studies evaluating artificial intelligence (AI) applications in interproximal caries detection on bitewing radiographs.

AI Model	Advantages	Disadvantages
YOLO (Object Detection CNN)	High accuracy and specificity for interproximal caries detection; fast real-time processing.	May struggle with smaller or early-stage lesions; requires large datasets for training.
U-Net (Semantic Segmentation CNN)	Effective segmentation of advanced lesions; precise localization of carious areas.	May not generalize well across different imaging modalities; performance dependent on dataset quality.
Mask R-CNN (Instance Segmentation CNN)	Combines object detection with segmentation; accurately outlines multiple lesions.	Computationally expensive; slower than simpler segmentation models like U-Net.
Faster R-CNN (Object Detection CNN)	Balanced diagnostic performance; strong accuracy and specificity for proximal caries.	Requires extensive labeled data; computationally expensive.
ResNet (Classification CNN)	Robust feature extraction; stable performance across different imaging datasets.	High computational requirements; less efficient for segmentation tasks.
Inception-ResNet (Hybrid Classification CNN)	Handles diverse clinical datasets well; strong feature extraction for complex tasks.	Complex architecture may increase processing time; interpretability challenges.
EfficientDet-Lite (Lightweight Object Detection CNN)	Optimized for real-time, resource-limited applications; portable and efficient.	Lower accuracy compared to more advanced models; may need refinement for clinical use.
VGG-16 (Classification CNN)	Good baseline model for classification; well-studied and reliable.	Less efficient compared to modern architectures; higher computational cost.
AlexNet (Classification CNN)	High diagnostic accuracy; strong performance in caries and restoration detection.	High computational requirements; less specialized for segmentation tasks.

Table 2. Comparison of advantages and disadvantages of different AI Models for Caries Detection.

Discussion

This review examined 25 studies that used different AI models to detect dental caries, showing significant improvements in diagnostic accuracy. The YOLO models performed well, with one achieving 94.59% accuracy and 98.19% specificity in detecting interproximal caries from 1,000 bitewing radiographs. Faster R-CNN and Inception-ResNet-v2 provided balanced diagnostic performance, with 87% accuracy, 86% precision, and 86% specificity on 2,468 bitewings. The U-Net model, applied to 3,686 bitewings, effectively segmented complex lesions with 80% accuracy and 83% specificity, while Mask R-CNN showed an F1-score of 68.9% to 71.9%, reinforcing its segmentation capabilities. Among classification models, AlexNet attained 90.30% accuracy, demonstrating strong diagnostic potential, while ResNet-based models exhibited 71.11% accuracy across different architectures. Overall, object detection models excelled in high-accuracy detection and segmentation models providing precise lesion localization.

These findings highlight AI's potential to improve diagnostic precision, streamline workflows, and increase access to consistent dental care, particularly in underserved areas. By integrating AI into dental diagnostics, we could reduce variations between clinicians and enhance early detection. This could have a significant impact on public health by standardizing diagnostic quality and expanding access to care, ultimately benefiting patients in regions with limited resources.

Conclusion

AI has the potential to revolutionize dental diagnostics by improving early detection and reducing inter-clinician variability. Its integration into public health systems can help address healthcare disparities by standardizing diagnostic accuracy and increasing access to care in underserved areas. Portable diagnostic tools, such as EfficientDet-Lite1, offer opportunities for enhancing preventive strategies and reducing treatment costs. However, challenges remain, including the need for diverse datasets, addressing ethical concerns related to transparency, and ensuring AI serves as a complement to, rather than a replacement for, clinical expertise. Future research should focus on the real-world implementation of AI, expanding datasets, and evaluating long-term outcomes to fully realize its potential in advancing oral healthcare delivery.

References



Introduction

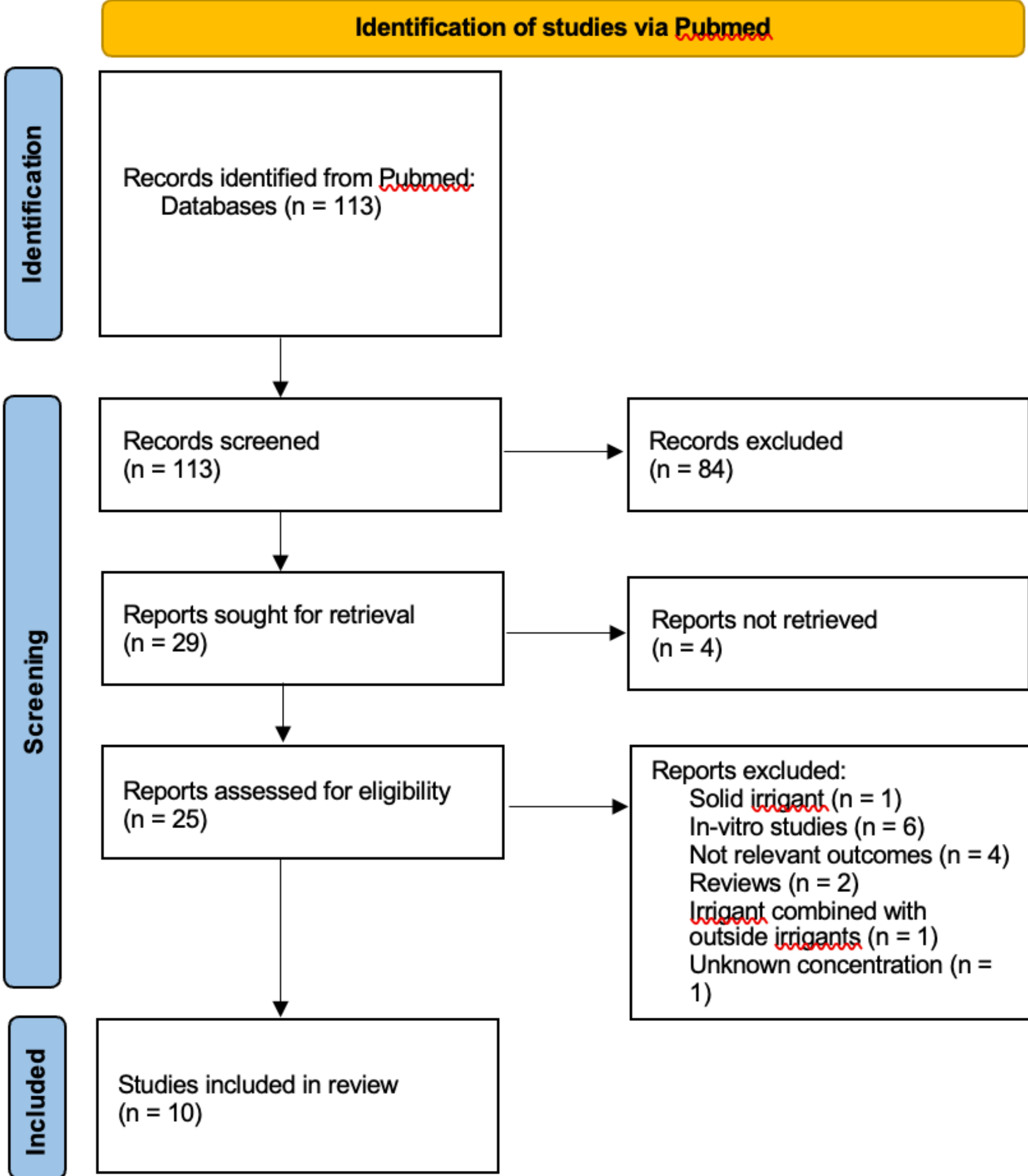
The oral cavity is the second-largest microbiome in the body containing bacteria known to cause periodontitis and oral cancer. Endodontic treatment aims to eliminate these bacteria within canals through irrigation like sodium hypochlorite (NaOCl), chlorhexidine (CHX), or ozone. NaOCl and CHX are widely used due to their bactericidal properties, but are cytotoxic, while ozone has fewer side effects but is less stable and effective in deep dentinal tubules. Research remains insufficient regarding the combined effectiveness of multiple irrigants and their antibacterial potential, prompting further studies to address these gaps of knowledge.

Methods

Study Design: Systematic review following Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) during January, 2025

- Inclusion Criteria: Clinical studies (randomized controlled trials, cohort, case-control, cross-sectional, case reports), published in English between January, 2000 and January, 2025.
- Exclusion criteria: studies published in other languages, inaccessible full text, outcomes other than antibacterial effects or side effects, and unknown concentrations.
- Search index: PubMed

Figure 1:



The PRISMA flow diagram illustrates the study selection process. It begins with 113 records identified from PubMed. 84 records were excluded, leaving 29 reports sought for retrieval. 4 reports were not retrieved, leaving 25 reports assessed for eligibility. 10 studies were included in the review. Exclusions were based on being solid irrigant (n=1), in-vitro studies (n=6), not relevant outcomes (n=4), reviews (n=2), irrigant combined with outside irrigants (n=1), and unknown concentration (n=1).

Mechanisms

1)NaOCl: NaOCl disinfects the canals by breaking down lipids into fatty acids and glycerol (saponification), increasing permeability, neutralization (oxidizing amino acids, denaturing proteins, and reducing pH), and lastly, chlorination (forming chloramines that damage bacterial DNA). The reactions degrade the bacterial cell wall, disrupt enzymes, and dissolve pulp tissue, eliminating biofilm.

2)CHX: CHX interacts with negatively charged bacterial membranes, destabilizing the cell wall, increasing permeability, and causing cytolysis. It can be bacteriostatic at low concentrations or bactericidal at high concentrations and is effective against gram-positive bacteria, though less efficient against gram-negative bacteria due to their outer membranes. It can be bacteriostatic at low concentrations or bactericidal at high concentrations and is effective against gram-positive bacteria, though less efficient against gram-negative bacteria due to their outer membranes.

3)Ozone: Ozone oxidizes bacterial cell walls, disrupting lipid layers and peptidoglycan structures, leading to osmotic lysis and intracellular leakage. It denatures proteins by altering thiol groups, interferes with DNA/RNA by breaking carbon-nitrogen bonds, and degrades extracellular polymeric substances (EPS), thereby preventing microbial survival.

Scheme 1. Saponification reaction.

Scheme 2. Amino acid neutralization reaction.

Scheme 3. Chloramination reaction.

Discussion

NaOCl remains the most effective irrigant for dissolving biofilms and eliminating bacteria. Combination treatments with ozone and higher concentrations of NaOCl increase its effectiveness. CHX demonstrates good antimicrobial substantivity but is less effective for biofilm removal, tissue degradation, and gram-negative bacteria. Ozone, while non-invasive and minimally toxic compared to the others, has limitations in deeper penetration and consistency when used alone. Combination treatments were shown to improve effectiveness, particularly those involving the use of high concentration NaOCl and ozone therapy.

Conclusions

To balance effectiveness and safety, the proposed protocol is to use 2.5% NaOCl at higher temperatures, followed by saline rinsing and liquid ozone to reduce residual bacteria. Future research should aim to refine this use of irrigant combinations and assess their long-term antibacterial efficacy and safety to establish more definitive clinical guidelines.

Study	NaOCl	CHX	Ozone	Combination	Results	Side Effects
Valera et al. 2015	2%, 2.5%	2%	N/A	N/A	2% NaOCl most effective in eliminating bacteria and preventing regrowth.	N/A
Zhang et al. 2015	N/A	2%	N/A	N/A	CHX had strongest residual antibacterial activity for 36 hours and effective against <i>E. faecalis</i> .	N/A
Ajeti et al. 2018	2.5%	2%	Unknown concentration used	2% CHX + ozone	Ozone showed significant improvement in elimination of aerobic and anaerobic bacteria colonies.	N/A
Plutzer et al. 2018	4%	0.2%	N/A	N/A	NaOCl had full bacterial elimination and CHX reduced bacterial numbers by 97%.	N/A
Ioannidis et al. 2018	2.5%	N/A	N/A	N/A	N/A	Volatile compounds emerge from interaction of NaOCl and infectious content inside canals. Chlorinated hydrocarbons (chloroform) and acetonitrile emerged.
Briseño-Marroquín et al. 2022	1%,3%,5%	0.12%, 0.2%, 2%	N/A	N/A	5% NaOCl showed largest inhibition zone diameter against E. faecalis, and increases in concentration for all irrigants increased with concentration increase. 2% CHX produces the largest inhibition zone against P.micro.	N/A
Tanvir et al. 2023	2.5%	N/A	N/A	N/A	NaOCl had a significant bactericidal effect against anaerobic microbial colonies.	N/A
Dede et al. 2023	3%	2%	N/A	2% CHX+3% NaOCl,	3% NaOCl (both combination and separately) has been able to decontaminate deeper dentine zones within the canals.	Dentinal surface was significantly altered with 3% NaOCl.
Kotecha et al. 2023	5.25% aqueous, 5.25 % gel	N/A	N/A	N/A	Both aqueus and gel NaOCl demonstrated bactericidal effects and difference between results for the two was insignificant.	N/A
Merçon et al. 2023	2.5%	N/A	8 µg/mL ozonated water	2.5% NaOCl and 8 µg/mL ozonated water	Combination of both irrigants showed significant reduction in bacterial counts in the biofilm.	N/A