Dentistry: A Multidisciplinary Approach

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About the Editors

Giuseppe Minervini

Giuseppe Minervini graduated in Dental Medicine in July 2016 with honors. During his undergraduate studies, he participated in an Erasmus project at "Rey Juan Carlos Alcorcon" in Madrid, Spain, from September 2013 to June 2014. He received his Postgraduate Diploma in Orthodontics in December 2020 from the University of Campania, Luigi Vanvitelli, Naples, Italy, and later earned his PhD at the same institution (XXXIV cycle). In 2019, he attended the Tweed Study Course at the Charles H. Tweed International Foundation in Tucson, Arizona. Currently, he serves as an Adjunct Professor at Saveetha Dental College and Hospitals, Saveetha University, Chennai. Dr. Minervini is recognized as one of the top 10 experts worldwide in the field of "Temporomandibular disorders" according to Scopus' rankings. He is a Subject Expert in Dental Materials and a Tutor at the Orthodontics Dentistry School at the University of Campania, Luigi Vanvitelli. His contributions include serving as an Editor for numerous dentistry journals (10 Editorial Boards, 14 Special Issues), and he is an active member of SIDO, EOS, and GSID. With over 188 publications, 70 posters, and an h-index of 32, he is a frequent speaker at national and international conferences. Dr. Minervini has received several awards for his contributions to the field. His research interests include biomedical and biomaterial applications in craniofacial, oral, and temporomandibular districts, as well as orthodontics, orofacial pain, temporomandibular joint disorders, and telemedicine.

Stefania Moccia

Stefania Moccia graduated in Pharmacy (University of Salerno, Italy) in 2013 with 110/110 cum laude. In 2017, she received her PhD in Pharmaceutical Sciences (University of Salerno, Italy) with the project "Novel functional foods from local Italian cultivars for consumers' well-being: from agricultural raw materials to design and development". Since 2014, she has been working at the Institute of Food Sciences (ISA-CNR) as a Contractor, and since 2019, she has worked as a full-time CNR Researcher for the strategic area "Food Production and Nutrition". Her research activity is mainly based on the extraction and characterization of bioactive compounds, in particular polyphenols and carotenoids, from food matrices, on the design and formulation of nutraceuticals, including the development of nanoemulsions, nanoparticles, and delivery systems, and the subsequent evaluation of their biological activity in preclinical models (cell lines and blood samples). In particular, she studies the chemopreventive and antioxidant activity of bioactive compounds (extracts/ purified fractions) from agri-food matrices for their functional characterization to identify the cellular processes involved and the markers of oxidative stress and aging.

Preface

Dear Colleagues,

This Special Issue presents a comprehensive collection of studies showcasing the latest innovations in dentistry, emphasizing the integration of multidisciplinary approaches and technological advancements. With a focus on improving patient outcomes and advancing oral health care, the contributions explore a range of topics, including the development of new biomaterials, minimally invasive techniques, and novel treatment protocols. These innovations have broad applications in restorative dentistry, prosthodontics, oral surgery, implantology, pediatric dentistry, orthodontics, and the management of temporomandibular disorders. The research presented in this reprint reflects the significant progress made in dental science and its application to clinical practice, offering valuable insights for both researchers and clinicians. Special acknowledgment is given to the contributing authors for their valuable insights, as well as to the editors and reviewers for their guidance throughout the process.

Giuseppe Minervini and Stefania Moccia
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Editorial

Dentistry: A Multidisciplinary Approach

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In this special issue of Medicina, we delve into the dynamic and ever-evolving world of dentistry, highlighting the remarkable innovations that are shaping the future of oral health and clinical dentistry practice. The articles featured in this issue underscore a critical shift in the dental field: the movement towards a multidisciplinary, technology-driven approach that touches upon various branches, including restorative dentistry, prosthodontics, oral surgery, implantology, pediatric dentistry, orthodontics, and the management of temporomandibular disorders.

The fusion of traditional dental practices with cutting-edge technology is not merely a trend; it is a paradigm shift in how we approach oral health. The advent of new biomaterials, digital modeling, and advanced surgical techniques has revolutionized the way dental professionals diagnose, treat, and manage dental and oral conditions [1]. The articles within this issue also emphasize the growing importance of personalized dental care. Advancements in biomaterials and surgical techniques allow for treatments that are tailored to the unique needs and conditions of each patient. This individualized approach is crucial, especially in complex cases where a standardized treatment may not suffice [2]. Furthermore, the exploration of new frontiers, such as the use of ozonated water for the treatment of Candida infections, represents a significant step towards finding more effective, safer, and less invasive treatment options. This not only enhances patient comfort and recovery but also opens up new avenues for treating a range of dental diseases [3].

Importantly, this issue highlights the critical role of ongoing research and education in the field of dentistry. The advancements we witness today are the results of relentless inquiry and learning [4]. As such, it is imperative for dental professionals to continue engaging in lifelong learning and to remain abreast of the latest developments in their field. This commitment to education and research is what will continue to drive the field forward, ensuring that dental care remains at the forefront of medical science and technology [5,6].

In summary, "Dentistry: A Multidisciplinary Approach" offers a comprehensive overview of the current state and future potential of dental care. It exemplifies how embracing a multidisciplinary, technologically advanced, and patient-centric approach can lead to significant advancements in all branches of dentistry. As we continue to expand the boundaries of what is possible in dental care, it is these principles that will guide us towards a future where oral health is integral to overall health and well-being. These changes have been seen especially in the fields of digital dentistry, tele-dentistry, and TMD treatment [7–10]. In the field of dentistry, recent advancements emphasize a holistic, interdisciplinary approach to treatment, particularly in complex cases. Studies have shown the efficacy of techniques like diagnostic mock-ups for crown lengthening, finite element analysis for bone stress assessment, and innovative approaches for treating malocclusions and cleft lip and palate.

The article "Soft Tissue Grafting Procedures before Restorations in the Esthetic Zone: A Minimally Invasive Interdisciplinary Case Report" presents a case study of a 32-year-old male patient with esthetic concerns regarding his anterior teeth. The patient exhibited generalized clinical attachment loss, gingival recessions, and cervical non-carious lesions. The treatment plan involved plastic mucogingival surgery using tunneling connective tissue grafts and anterior ceramic laminate veneers. The surgical approach focused on improving root coverage and gingival architecture, while the restorative phase aimed to enhance dental esthetics with veneers. The case highlights the importance of an interdisciplinary approach, combining periodontal and restorative treatments, to achieve satisfying esthetic outcomes in complex cases [11]. "The Stability Guided Multidisciplinary Treatment of Skeletal Class III Malocclusion Involving Impacted Canines and Thin Periodontal Biotype" presents a case study of a 16-year-old female patient with dental and skeletal Class III malocclusion, bilaterally impacted maxillary canines, and a thin gingival biotype. The treatment involved orthognathic surgery, subepithelial connective tissue graft surgery, and a segmental arch technique. The study emphasizes the importance of a multidisciplinary approach to addressing complex dental and skeletal issues, highlighting the role of periodontal management in ensuring long-term stability and aesthetic success [12]. The article "Evaluation of the Sensitivity of Selected Candida Strains to Ozonated Water—An In Vitro Study" investigates the sensitivity of Candida strains to ozonated water. The study evaluated the impact of ozonated water at varying concentrations and exposure times on *Candida albicans*, *Candida glabrata*, and *Candida krusei strains*. The findings indicated that all the strains were sensitive to ozonated water, with increased sensitivity correlating with higher concentrations and longer exposure times. The effectiveness of ozonated water against these Candida strains was comparable to 0.2% chlorhexidine gluconate, suggesting its potential as an effective alternative for oral candidiasis treatment [3]. "Diagnostic Mock-Up as a Surgical Reduction Guide for Crown Lengthening: Technique Description and Case Report" discusses a technique using a diagnostic mock-up as a guide for crown-lengthening surgery to improve gingival architecture. This method was applied to a 30-year-old female patient concerned about her "gummy smile" and short clinical crowns. The process involved a diagnostic wax-up, a provisional overlay for surgical guidance, and final restorations with ceramic crowns and veneers. The study highlights the advantages of this technique in achieving desired aesthetic outcomes in complex dental cases [13]. The article "Injectable Resin Technique as a Restorative Alternative in a Cleft Lip and Palate Patient: A Case Report" details the treatment of a 21-year-old female patient with a unilateral left cleft lip and palate. It focuses on the use of an injectable composite resin technique for dental re-anatomization, offering a minimally invasive, efficient, and aesthetically pleasing option. This technique allowed for the successful restoration of the patient's teeth, improving her dental anatomy and aesthetics, with positive results observed after one year [14]. The article "Neural Basis of Etiopathogenesis and Treatment of Cervicogenic Orofacial Pain" discusses the neuroanatomical and neurophysiological basis of cervicogenic pain in cervico-cranial pain syndromes, with a focus on cervico-orofacial syndromes. It covers a wide range of topics, including the clinical anatomy of the cervico-cranial junction, the role of the temporomandibular joint, and the integrative function of the cervico-cranial complex. The article emphasizes the importance of understanding neuroanatomical and neurophysiological neuromuscular relations for effective therapeutic approaches, which are primarily based on orthopedic manual and dental occlusal treatment [15]. The article "Cortical and Trabecular Bone Stress Assessment during Periodontal Breakdown–A Comparative Finite Element Analysis of Multiple Failure Criteria" by Radu Andrei Moga et al. [16] presents a numerical analysis exploring the biomechanical behavior of the mandibular bone under orthodontic forces during periodontal breakdown. It evaluates the appropriateness of various failure criteria (Von Mises, Tresca, maximum/minimum principal stresses, and hydrostatic pressure) for studying bone under these conditions. The study involves 405 simulations across 81 mandibular models with varying levels of bone loss and orthodontic movements (intrusion, extrusion, tipping, rotation, and translation). The results show that Tresca and Von Mises criteria are most suitable for bone stress analysis, displaying a coherent pattern of increasing stress across all movements and levels of periodontal breakdown. The study concludes that Tresca is better suited as a unified criterion for the study of teeth and surrounding periodontium [16]. The article "Modular Digital and

3D-Printed Dental Models with Applicability in Dental Education" explores the impact of digitalization in dental education. It discusses the development and use of modular digital dental models and 3D-printed models in teaching. The study assesses the opinions of dental students regarding these methods, emphasizing their benefits in enhancing practical skills and the understanding of dental procedures. This reflects a significant shift towards integrating advanced technology in dental education, aiming to improve student learning experiences and outcomes [17]. The article "Full-Mouth Rehabilitation of a Patient with Gummy Smile—Multidisciplinary Approach: Case Report" in Medicina describes the comprehensive treatment of a 48-year-old female patient with aesthetic concerns and disturbed masticatory function due to missing posterior teeth and a gummy smile. The treatment plan involved advanced techniques such as diode laser and piezo-surgery, implant installation, and the use of zirconia ceramic for final restorations. This multidisciplinary approach, spanning over two years, significantly improved the patient's dental function and aesthetics. This case underscores the importance of personalized, multifaceted treatment strategies in complex dental cases [18]. The article "Evaluation of Clinical and Oral Findings in Patients with Epidermolysis Bullosa" in Medicina focuses on the oral and dental manifestations in patients with Epidermolysis Bullosa (EB), a genetic skin disorder. The study involves an assessment of clinical and oral findings in 26 EB patients, highlighting various complications like dental caries, enamel hypoplasia, and oral lesions. It underscores the unique dental care requirements of EB patients and suggests the need for specialized treatment approaches [19]. The article "A Comparative Analysis of Dental Measurements in Physical and Digital Orthodontic Case Study Models" by Elena-Raluca Baciu et al. [20] compares manual and digital orthodontic measurements on both physical and digital models. The study aims to determine the reliability of digital models in orthodontic analyses, focusing on the reproducibility of dental arch characteristics. It involves a detailed comparison of different measurement techniques applied to various types of models, including physical models created through traditional pouring and additive manufacturing as well as digital models obtained through scanning. The research concludes that both traditional and digital models are effective for orthodontic teaching, with no significant differences in the measurement results [20]. The article "The Impact of Simulated Bruxism Forces and Surface Aging Treatments on Two Dental Nano-Biocomposites—A Radiographic and Tomographic Analysis" by Amelia Anita Boitor et al. [21] investigates the effects of simulated bruxism forces and aging treatments on two dental nano-biocomposites. It focuses on the radiographic and tomographic analysis of these materials under stress. The study simulates real-life conditions like the consumption of acidic beverages and the use of at-home dental bleaching, aiming to assess the mechanical and functional behavior of these composites under such circumstances. The results provide insights into the suitability of these materials for dental restorations in patients with specific oral conditions, including bruxism [21]. The study "Different Designs of Deep Marginal Elevation and Its Influence on Fracture Resistance of Teeth with Monolith Zirconia Full-Contour Crowns" by Ali Robaian et al. [22] investigates the impact of deep marginal elevation (DME) on the fracture resistance of teeth restored with monolithic zirconia crowns. Forty premolars were divided into four groups, each undergoing different preparation and restoration procedures. The study found that fracture resistance decreased with increasing tooth structure involvement, even with monolithic zirconia crowns. However, DME up to 2 mm below the cemento-enamel junction did not negatively influence fracture resistance, suggesting its viability in clinical scenarios. The study emphasizes the importance of considering tooth preservation and material choice in restorative dentistry [22]. The article "Cranial and Odontological Methods for Sex Estimation—A Scoping Review" by Laura Maria Beschiu et al. [23] provides a comprehensive review of various methods used for sex estimation based on cranial and dental records. The study covers articles published between January 2015 and July 2022, focusing on morphometric, morphologic, and biochemical analyses in living populations, autopsy cases, and archaeological records. The review highlights that cranial and odontological sex estimation methods are highly population-specific and underscores the need for these methods to be applied to and verified in more populations. It also emphasizes the high accuracy of DNA analysis while noting the limitations and challenges of other methods for predicting sex from cranial or odontological records [23]. The article "Comparison of Mechanical Properties of Three Tissue Conditioners: An Evaluation In Vitro Study" by Marcin Mikulewicz et al. [24] compares the mechanical properties of three tissue conditioners (TC) used in dentistry. It focuses on various properties like Shore A hardness, ethanol concentration, sorption, solubility, and adhesion to denture base, evaluated under specific test conditions. The study concludes that materials containing non-phthalate plasticizers showed higher solubility and increased hardness when stored in distilled water compared to those containing phthalates. It emphasizes the importance of understanding the properties of commercial TC for optimal clinical performance and highlights the need for further research to improve these materials, especially considering the use of phthalate-free alternatives [24]. The article "Assessment and Correlation of Salivary Ca, Mg, and pH in Smokers and Non-Smokers with Generalized Chronic Periodontitis" by Saad Mohammad Alqahtani et al. [25] investigates the relationship between salivary calcium, magnesium, pH levels, and periodontitis in smokers and non-smokers. The study, conducted on 210 individuals, reveals significant differences in salivary calcium levels between smokers and non-smokers with periodontitis. It suggests that higher salivary calcium levels in smokers could be a potential marker for periodontitis progression, emphasizing the role of saliva as a diagnostic tool in periodontal diseases [25]. The article "Misfit of Implant-Supported Zirconia (Y-TZP) CAD-CAM Framework Compared to Non-Zirconia Frameworks: A Systematic Review" by Hussain D. Alsayed [26] systematically reviews studies comparing the misfit of yttria-stabilized zirconia (Y-TZP) CAD-CAM implant-supported frameworks with other materials. It includes 11 articles and covers different methods like scanning electron microscopy, one-screw tests, and 3D virtual assessment. The findings suggest that Y-TZP CAD-CAM frameworks have comparable misfits to other materials. However, due to methodological heterogeneity, the numerical misfit values are debatable, highlighting the need for standardized and well-designed in vitro and clinical studies in order to obtain definitive conclusions [26]. The article "Gaucher: A Systematic Review on Oral and Radiological Aspects" by Giuseppe Minervini et al. [27] provides a systematic review of Gaucher disease, particularly focusing on its oral and radiological manifestations. It evaluates the principal findings in the jaw using cone-beam computed tomography and X-ray orthopantomography. The study underlines the importance of dental professionals in the early diagnosis and management of Gaucher disease, emphasizing the role of dental radiographs in detecting jawbone involvement, a common feature in Gaucher patients [27]. The article "Identification of the Remains of an Adult Using DNA from Their Deciduous Teeth as a Reference Sample" by María-de-Lourdes Chávez-Briones et al. [28] presents a unique forensic case. It details the identification of an adult's remains using DNA from their deciduous teeth, kept by the mother. This innovative approach proved crucial in this case, emphasizing the potential of using personal artifacts or saved biological samples as reference DNA in forensic investigations, especially in scenarios where conventional methods are insufficient [28]. The article "The Effect of Dentine Desensitizing Agents on the Retention of Cemented Fixed Dental Prostheses: A Systematic Review" by Mohammed E. Sayed [29] examines the impact of dentine desensitizing agents on the retention of cemented fixed dental prostheses. This systematic review compiles and analyzes data from various studies to determine how these agents affect retention. It evaluates multiple types of desensitizing agents and their interactions with different luting cements. The findings are crucial for clinical decision-making, offering guidance on selecting appropriate desensitizing agents to ensure the optimal retention of dental prostheses [29]. The article "The Impact of Anemia-Related Early Childhood Caries on Parents' and Children's Quality of Life" by Dila Özyılkan et al. [30] explores the relationship between anemia-related dental caries in children and their quality of life, as well as that of their parents. Utilizing the Early Childhood Oral Health Impact Scale (ECOHIS) and the Parental-Caregivers Perceptions Questionnaire (P-CPQ), the study assesses the impact of these dental issues on children and parents. The findings highlight the significant negative impact of anemia-related dental caries on quality of life, underscoring the importance of prioritizing preventive measures and timely dental treatments for affected children [30].

Conflicts of Interest: The author declares no conflicts of interest.

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Case Report

Identification of the Remains of an Adult Using DNA from Their Deciduous Teeth as a Reference Sample

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Abstract: In many forensic cases, the identification of human remains is performed by comparing their genetic profile with profiles from reference samples of relatives, usually the parents. Here, we report, for the first time, the identification of the remains of an adult using DNA from the person's deciduous teeth as a reference sample. Fragments of a skeletonized and burned body were found, and a short tandem repeat (STR) profile was obtained. A woman looking for her missing son went to the authorities. When the DNA profile of the woman was compared to a database, a positive match suggested a first-degree kinship with the person to whom the remains belonged. The woman had kept three deciduous molars from her son for more than thirty years. DNA typing of dental pulp was performed. The genetic profiles obtained from the molars and those from the remains coincided in all alleles. The random match probability was 1 in 2.70×10^{21}. Thus, the remains were fully identified. In the routine identification of human remains, ambiguous STR results may occur due to the presence of null alleles or other mutational events. In addition, erroneous results can be produced by false matches with close family members or even with people who are completely unrelated to the victim, such that, in some cases, a probability of paternity greater than 99.99% does not necessarily indicate biological paternity. Whenever possible, it is preferable to use reference samples from the putative victim as a source of DNA for identification.

Keywords: dental pulp; DNA analysis; human remains; reference sample; teeth; victim identification

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1. Introduction

One of the main tasks of legal systems in the investigation of criminal cases is the personal identification of unknown human remains. To achieve this purpose, collaboration between forensic anthropologists, pathologists, and odontologists may be crucial. Also, DNA profiling can be used in the identification of skeletonized or highly decomposed human remains. Identification is usually carried out by comparing the genetic profile from the remains with the genotypes of reference samples from relatives, most commonly the parents of the victim. However, in these cases, ambiguous results may occur due to the presence of null alleles or other mutational events, and erroneous results can be produced by false matches with close family members or even with people who are completely unrelated to the victim [1,2].

Thus, in the identification of human remains by DNA typing, it would be ideal to use biological samples of the person from whom the remains are suspected to have come as a reference. However, there are few reports in the literature on the successful use of this strategy. Calacal et al. [3] identified the skeletal remains of two children by directly comparing the genetic profiles derived from the remains with the profiles from children's umbilical tissues, which had been preserved by their mothers. Tanaka et al. [4] identified two corpses in two criminal cases using the toothbrushes of the victims as DNA sources.

Sweet et al. [5] identified a skeleton using a reference sample consisting of cytological smears stained with the Papanicolaou method, obtained from the medical record of the deceased. Other studies have analyzed the feasibility of using objects or samples from the victim, such as cosmetic applicators [6] and archived tumor samples [7], to obtain DNA that can be used to identify human remains; however, these strategies have not been applied in actual criminal cases.

While bloodstains or buccal swabs would be the perfect reference samples from the victim for the identification of unknown remains, they are often not available. Here, we report, for the first time, the use of DNA isolated from deciduous teeth as a reference sample to identify an adult victim in an actual criminal case.

2. Case Presentation

Fragments of a skeletonized and burned body were found on the slopes of a hill. Four of the least damaged bone fragments were selected for DNA extraction. Given the physical condition of the body, we could neither determine to which bones the analyzed fragments belonged nor characteristics such as the sex or the approximate age of the deceased. The outer surfaces of the fragments were cleaned by immersion in 50% commercial bleach for 15 min. Next, they were washed briefly with nuclease-free water (5 washes), then immersed briefly in 100% ethanol and air-dried overnight in a sterile hood. The samples were frozen with liquid nitrogen and pulverized with a pestle and mortar. The bone powder (0.5 g) was decalcified by incubating it with a 0.5 M EDTA solution on a rocking platform at 37 °C for 5 days with three solution changes. Samples were centrifuged, and the pellets were rinsed twice in double-distilled water. DNA extraction was performed using the PrepFiler Express BTA™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA, USA). Lysis buffer from the kit was added to the samples together with 1 M DTT and Proteinase K (2 mg/mL). Samples were incubated overnight in a thermal shaker at 56 °C. Finally, they were centrifuged, and the supernatant was subjected to DNA extraction in the AutoMate™ Instrument (Applied Biosystems) following the manufacturer's instructions. The DNA samples were quantified on the 7500 ABI Real-Time PCR platform using the Quantifiler Trio DNA quantification kit (Applied Biosystems). All samples had DNA concentrations > 0.01 ng/µL and were therefore deemed suitable for DNA typing [8].

DNA typing was carried out using the commercially available multiplex kit AmpFℓSTR® Identifiler Plus (Applied Biosystems), following the protocol provided by the manufacturer. In an attempt to ensure the amplification of as many alleles as possible, the samples were also amplified by the AmpFℓSTR® MiniFiler kit (Applied Biosystems), which has nine loci in common with the previous kit. Capillary electrophoresis was performed in an ABI PRISM® 310 genetic analyzer (Applied Biosystems). Samples were run on a capillary containing POP-4 polymer; allele assignment was determined by comparison with allelic ladders included in the kits, and genotypes were generated using GeneMapper® IDX-v1.4 software (Applied Biosystems).

No alleles from bone fragment number 1 were amplified. Partial consensus profiles (combining the results of both kits) were obtained from fragments 2 and 3. A complete consensus profile was obtained from fragment 4. The genetic profile obtained was stored in a database containing genotypes from unidentified cadavers.

Four years later, a woman looking for her missing son went to the authorities. A saliva sample was obtained according to the usual protocol followed in these cases at our institution. DNA was extracted from the sample using a Chelex protocol [9]. DNA typing was performed as described above for the bone fragments, but only with the Identifiler kit. When the woman's DNA profile was compared to the database, a positive match suggested a first-degree kinship with the person to whom the remains belonged. The woman was asked about the existence of other first-degree relatives of her son, which could allow a complete identification of the remains. She denied the availability of the father and other first-degree relatives of her son. Later in the interview, she recalled that she had kept three deciduous molars from her son in a plastic bag for more than thirty years (Figure 1). She was asked for the molars to see whether they could serve as a reference sample.

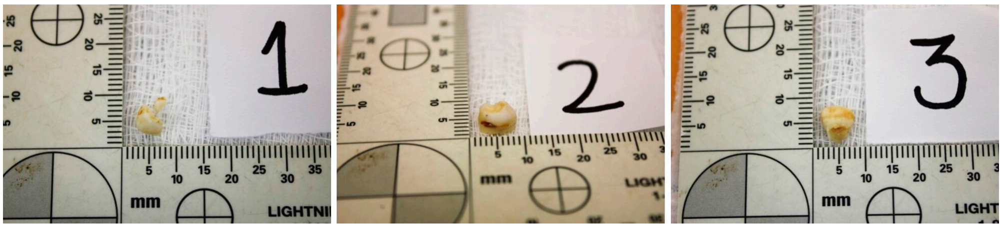

Figure 1. Deciduous molars submitted for DNA typing.

Dental pulp tissue was collected from each molar by sectioning using a carborundum disc. DNA was isolated by Proteinase K digestion and phenol chloroform extraction methods [10] and quantified as described for the bone fragments. DNA typing was performed with the Identifiler and MiniFiler kits as described above. A complete consensus profile was obtained from molar 1. A partial consensus profile was obtained from molar 2. No alleles from molar 3 were amplified.

All the genetic profiles generated are presented in Table 1. The genetic profiles obtained from the bone fragments and the molars coincided in all the alleles. Every locus was sequenced from the bone fragments, and the molars shared at least one allele with the corresponding locus generated from the putative mother. The random match probability and the probability of parentage were calculated using STR allele frequency data from our population and PATPCR software version 2.0.2 [11,12]. The random match probability was 1 in 2.70×10^{21}, and the probability of parentage was 99.9999%. Thus, the remains were fully identified and returned to the victim's biological mother.

Table 1. Comparison of short tandem repeats results of DNA recovered from bone fragments, deciduous molars, and the alleged mother of the victim.

Locus	Bone Fragments	Deciduous Molars	Alleged Mother
Amelogenin	XY	XY	XX
D8S1179	14, 15	14, 15	12, 14
D21S11	32.2, 33.2	32.2, 33.2	29, 33.2
D7S820	10, 10	10, 10	10, 10
CSF1PO	10, 11	10, 11	11, 11
D3S1358	15, 18	15, 18	14, 15
TH01	6, 6	6, 6	6, 6
D13S317	9, 14	9, 14	9, 9
D16S539	11, 12	11, 12	11, 12
D2S1338	18, 25	18, 25	23, 25
D19S433	11.2, 13	11.2, 13	11.2, 15
vWA	16, 17	16, 17	16, 17
TPOX	9, 12	9, 12	8, 9
D18S51	12, 15	12, 15	15, 16
D5S818	11, 12	11, 12	11, 11
FGA	21, 24	21, 24	24, 24

3. Discussion

Dental pulp is a rich source of DNA amenable to genetic analysis; the latter can be used for the positive identification of human remains, especially when soft tissue destruction has occurred. DNA analysis is usually carried out by comparing the genetic profile of the teeth from the remains with the genotypes of reference samples from relatives, most commonly the parents of the victim. For purposes such as crime solving, missing-person cases, and disaster victim identification, this approach has been used for decades [13]. However, to our knowledge, this is the first report of the use of DNA isolated from teeth as a reference sample to identify a victim in a criminal case.

In the case presented here, unambiguous identification was achieved thanks to the matching of DNA profiles generated from the bone fragments with those from the teeth. The DNA profile from the mother served to reinforce the results.

Short tandem repeats (STRs) are the most widely used genetic markers for human identity determination and paternity testing. Their use makes it possible to clarify most legal and forensic cases with a generally very high degree of certainty [14]. As mentioned above, the identification of human remains is generally performed by comparing the genetic profile of the remains with that of first-degree relatives, usually the parents. However, ambiguous STR results may occur due to the presence of null alleles or other mutational events (for specific cases, see [15–28]; for studies in populations, see [29–37]). STRs have mutation rates ranging from 0 to 7×10^{-3}, with an average of 2×10^{-3} [33,34]. The most frequent mechanism causing these mutations is the slippage of the DNA replication complex during DNA synthesis [30]. In the most common mutations, an STR differs only slightly in its size from its presumed predecessor. The gain or loss of tandem repeats could lead to false maternal or paternal exclusions [30,32]. In addition, erroneous results can be produced by false matches with close family members or even with people who are completely unrelated to the victim, such that, in some cases, a probability of paternity greater than 99.99% does not necessarily indicate biological paternity [30,31,38–41]. Poetsch et al. [31] investigated how many wrong paternity inclusions could be detected when comparing 13-15 STRs between 336 children and 348 unrelated men. They found that at least one and up to three "second father(s)" could be found for 23 children. In general, the false inclusion rate ranges between 19% and 23% [40]. These problems are being reported more frequently and are most common in cases where only one putative parent is available [31,40,42]. The inclusion of additional autosomal STR loci may assist in clarifying some ambiguous cases. Sometimes, however, the addition of more loci introduces additional mismatches. Furthermore, it has been observed that the inclusion of more loci does not compensate for the absence of genetic information from the mother or the father [35,40,42–44]. The use of Y-chromosome STRs can help only when the victim is male, and the possibility that a close relative of the putative father is the biological father cannot be ruled out [45]. X-chromosome STRs must be analyzed along with other genetic markers to obtain useful data and can only be used with accuracy when the victim is female, as there are no X-chromosome alleles inherited by descent in a father-son relationship [46]. Other typing systems that may be used to resolve ambiguous cases include the HV1 and HV2 hypervariable regions of mitochondrial DNA, single nucleotide polymorphisms (SNPs), and next-generation sequencing (NGS). However, they are expensive and time-consuming and are not available in most developing countries [47]. Even with these systems, the lack of informative reference samples (first-degree relatives) is the most common problem in identifying unknown corpses [41,48]. Thus, whenever possible, it is preferable to use reference samples from the putative victim as a source of DNA for identification.

In this study, we analyzed three deciduous molars. A complete DNA profile was obtained from only one molar. The efficiency of DNA typing from teeth subjected to various experimental conditions, such as treatment with acids [49] and fire exposure [50,51], has been reported in the literature. In addition, the effect of the duration of the postmortem and postextraction periods in obtaining genetic profiles from the teeth has been analyzed [52,53]. From these and other studies, it can be concluded that the usefulness of teeth to obtain a genetic profile not only depends on the conditions to which they are subjected before analysis but also varies between individuals and even within the same individual. This inter- and intra-individual variation may be due to a wide difference in the number of cells present in each individual tooth, resulting in a different DNA yield. In turn, the different number of cells is due to various factors, including the presence or absence of disease and the age of the subject. Therefore, each identification case must be considered individually [53,54].

It is important to note that the mother's decision to keep some teeth from her son was essential for the resolution of this case. This and similar practices [55] should be promoted, as teeth can be an alternative source of reference DNA for the identification of persons in, for example, mass disasters or criminal cases. Other samples may also be considered for this purpose, such as buccal swabs, hair, and blood spots. Instructions for their collection and preservation, as well as the material required even in a domestic setting, can be easily found on the Internet. However, in this regard, one must be very careful and sensitive and respect the customs and beliefs of a particular society or individual.

Finally, although DNA profiling is an important element for the identification of human remains, several factors can affect the results of this analysis, such as an insufficient amount of extracted DNA or its degradation in cases of poorly preserved samples. In such cases, a multidisciplinary approach may be necessary that considers the use of other disciplines, including forensic anthropology and odontology [56,57].

4. Conclusions

This is the first reported case of the use of DNA isolated from teeth as a reference sample to identify a victim in a criminal case. Whenever possible, it is preferable to use reference samples from the putative victim as a source of DNA for identification.

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Article

Cortical and Trabecular Bone Stress Assessment during Periodontal Breakdown–A Comparative Finite Element Analysis of Multiple Failure Criteria

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Abstract: *Background and Objectives:* This numerical analysis investigated the biomechanical behavior of the mandibular bone as a structure subjected to 0.5 N of orthodontic force during periodontal breakdown. Additionally, the suitability of the five most used failure criteria (Von Mises (VM), Tresca (T), maximum principal (S1), minimum principal (S3), and hydrostatic pressure (HP)) for the study of bone was assessed, and a single criterion was identified for the study of teeth and the surrounding periodontium (by performing correlations with other FEA studies). *Materials and Methods:* The finite element analysis (FEA) employed 405 simulations over eighty-one mandibular models with variable levels of bone loss (0–8 mm) and five orthodontic movements (intrusion, extrusion, tipping, rotation, and translation). For the numerical analysis of bone, the ductile failure criteria are suitable (T and VM are adequate for the study of bone), with Tresca being more suited. S1, S3, and HP criteria, due to their distinctive design dedicated to brittle materials and liquids/gas, only occasionally correctly described the bone stress distribution. *Results:* Only T and VM displayed a coherent and correlated gradual stress increase pattern for all five movements and levels of the periodontal breakdown. The quantitative values provided by T and VM were the highest (for each movement and level of bone loss) among all five criteria. The MHP (maximum physiological hydrostatic pressure) was exceeded in all simulations since the mandibular bone is anatomically less vascularized, and the ischemic risks are reduced. Only T and VM displayed a correlated (both qualitative and quantitative) stress increase for all five movements. Both T and VM displayed rotation and translation, closely followed by tipping, as stressful movements, while intrusion and extrusion were less stressful for the mandibular bone. *Conclusions:* Based on correlations with earlier numerical studies on the same models and boundary conditions, T seems better suited as a single unitary failure criterion for the study of teeth and the surrounding periodontium.

Keywords: bone; bone loss; orthodontic force; finite element analysis; orthodontic movement

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1. Introduction

The bone structure and periodontal ligament (PDL) are the supporting tissues of the tooth and are subjected to various amounts and forms of stresses during orthodontic treatment [1,2]. Two types of bone can be distinguished: the cortical and the trabecular/cancellous bone, which, biomechanically, should be analyzed as a continuum [1–8]. The trabecular component holds the bone marrow and vascular vessels and has a higher regeneration potential than cortical bone [9]. Both components are anatomically anisotropic materials, with trabecular bone being a highly porous mineralized material while cortical bone is a highly mineralized compact structure [1]. Cortical bone has the function of structural support for surrounding dental tissues and the protection of trabecular/cancellous bone [5].

Bone as a continuum and as a single-stand structure possesses a high adaptation ability to alter its geometry to provide the strongest structure possible with a minimum amount of tissue [7]. The bone structure can also absorb and dissipate energy/stresses and elastically deform, preventing fracture and/or destruction [7]. Its internal structural micro-architecture allows for microcracks/damage (i.e., linear, and diffuse microcracks, and microfractures) and time to heal [7]. Linear microcracks appear as a response to compressive stresses (older age, more brittleness), the diffuse microdamage as a response to tensile stresses (younger age, more ductileness), while microfractures as a response to shear stresses (older age, mixed ductile brittleness, mostly in trabecular bone) [7]. Thus, from a biomechanical engineering perspective, the bone structural behavior depends on the applied loads acting as a ductile material with a certain brittle flow mode [2,7,10–12].

The orthodontic stresses from the tooth are transmitted through the PDL to the bone, while the display areas are also influenced by the tissular anatomy and integrity [8]. Periodontal breakdown is found in orthodontic patients, affecting the biomechanical behavior of the tooth and surrounding support tissues, with higher tissular amounts of stress appearing along with bone loss [2,10–12]. In the bone structure, Burr et al. [7] reported microcracks and microdamage near the resorption and remodeling areas and a decrease in fracture risks in their presence (due to internal micro-architectural changes), which influence the structural stress display. It must be emphasized that the biomechanical behavior in both the intact and reduced periodontium is multifactorial, which depends on the cortical and trabecular structural continuum, material, and structural properties [6].

No studies investigating the mandibular bone stress distribution in a gradual periodontal breakdown under orthodontic loads were found, despite multiple bone-implant FEA (finite element analysis) research studies with a focus on the implant and the surrounding bone [1,5,8,13–18].

Only three reports were found to assess the stress distribution both in the tooth and its intact surrounding support system (0.35–0.5 N of tipping [19,20]; 10 N of intrusion, 3 N of tipping and translation [21]); these showed various qualitative and quantitative results but no correlation with the maximum physiological hydrostatic pressure (MHP) and/or failure criteria type of the analyzed material. These reports employed both ductile (Von Mises) and brittle (maximum principal stress) failure criteria and supplied results that did not entirely match the clinical data.

The orthodontic biomechanical behavior of bone is influenced by the anatomy of tissues, materials, magnitude, and the quantity and quality of the bone [1,14]. There are several tools to analyze the biomechanical behavior of bone and teeth, including in vitro assays (photoelastic stress analysis, static/dynamic mechanical fracture tests) and numerical simulations (finite elements analysis) [1]. FEA is the only method that allows the individual analysis of each component of a structure, providing accurate results if the input data (anatomical accuracy and loading conditions) are correct [1,13]. Only a numerical simulation such as FEA allows for correct biomechanical studies that assess and predict stress distribution in living dental tissues [13,14,17,22].

FEA accuracy also depends on the selection of proper and adequate failure criteria. There are multiple failure criteria, each specially designed to better describe the biomechanical behavior of a certain type of material: brittle-maximum S1 tensile and minimum S3 compressive principal stresses, ductile-Von Mises (VM) overall/equivalent and Tresca (T) shear stress, and liquid/gas-hydrostatic pressure (HP) [2,10–12]. The main difference between these types of materials is related to the way they deform under loads (yielding materials theory) [2,10–12]. The ductile materials suffer from various forms of recoverable deformations, returning to their original form after the force effect has ceased [2,10–12]. The brittle materials, when subjected to various loads, suffer from various degrees of plastic non-recoverable deformations, with modification of their original shape and dimensions (necking and buckling effects) before their fracture and destruction [2,10–12]. Hydrostatic pressure describes a specific physical state where there is no shear stress (which is not adequate for solid materials as ductile and brittle) [2,10–12]. This approach, based on the assessment of material type when performing the FEA analysis, has not been found in other studies (except in our team's earlier studies [2,10–12]) despite its importance for the accuracy of results [22]. Moreover, there are no FEA studies of bone to compare various failure criteria and to select the most adequate one based on the results.

The dental tissues (dentine, cement, dental pulp, neurovascular bundle, PDL, bone, and stainless-steel bracket) are all considered to resemble ductile materials with a certain brittle flow mode [2,10–12]. Only enamel is a brittle material due to its internal micro-architecture [23,24]. Nevertheless, since it represents only an extremely small percentage of the entire volume of dental tissues, and the entire structure behaves as ductile, the adequate and acceptable failure criteria is that of ductile materials [2,10–12,17].

Bone, when subjected to internal stress, undergoes a certain amount of recoverable elastic deformations because of the PDL stress transmitted to the bone, beyond which microfractures appear and bone loss results [14]. According to the engineering composite beam theory, when materials with different elastic modulus (cortical and trabecular bone, PDL and dentine; Table 1) interact and are subjected to a load, the highest stress is located at the first point of contact (i.e., bone cervical third) [14]. Hooke's law states that the deformation of materials depends on their elastic modulus; the higher the modulus, the smaller the deformation [14,22]. In the tooth and surrounding support system, the periodontal ligament, followed by bone, suffers the highest deformation [10–12,14].

Table 1. Elastic properties of materials.

Material	Young's Modulus, E (GPa)	Poisson Ratio, υ	Refs.
Enamel	80	0.33	[2,10–12]
Dentin/Cementum	18.6	0.31	[2,10–12]
Pulp	0.0021	0.45	[2,10–12]
PDL	0.0667	0.49	[2,10–12]
Cortical bone	14.5	0.323	[2,10–12]
Trabecular bone	1.37	0.3	[2,10–12]
Bracket (Stainless Steel)	190	0.265	[2,10–12]

Most bone-implant FEA studies employed the adequate VM failure criteria in intact bone, reporting stress concentrations in the cortical component located in cervical third areas, while in the trabecular component, these occurred in a broader area [1,5,8,13–17,21]; however, they did not address the suitability issues (VM is more suited for homogeneous materials, while bone is non-homogenous). There are biomechanical reports suggesting that the shear stress produced by occlusal loadings contributes to bone resorption around the implants [13]. However, there were no studies found assessing the periodontal breakdown influence over the stress distribution in bone.

For avoiding ischemia, necrosis, and further periodontal loss, the physiological maximum hydrostatic pressure of 16 KPa [2,10–12] should not be exceeded, especially in the well-vascularized dental tissues and dental tissues that are easily deformable under stress (i.e., PDL, dental pulp, and the neuro-vascular bundle (NVB)). However, in the less deformable and vascularized tissues (i.e., dentine, bone), amounts of stress higher than the MHP could appear without significant tissue losses. However, these amounts of stress should not exceed the maximum tensile, shear, and compressive strength of each material (which never occurs in clinical daily practice).

Nevertheless, in orthodontic biomechanics, the PDL is the triggering factor for the orthodontic movements (due to circulatory disturbances) inducing bone remodeling. If these circulatory disturbances are severe and last for a longer period, the inevitable ischemia will lead to necrosis and tissue loss. Usually, in intact periodontium, the orthodontic forces from daily practice are light [25] and up to 1.5 N (approx. 150 gf) [2,10–12]. Nevertheless, there is little information about the orthodontic forces that can be safely applied in the reduced periodontium [10–12]. Earlier studies from our group reported for the PDL, dental pulp, and neurovascular bundle a reduction of applied forces for an 8 mm reduced periodontium to avoid exceeding the MHP. The areas of higher stress were reported to be the cervical third of PDL, with less stress in the apical third, where the NVB is found. Nevertheless, the issues of MHP and correlations with the highly vascularized dental tissues (as PDL, pulp, and NVB) should be approached in a bone study.

In the dental field, the FEA numerical method is well represented in many studies of PDL and implants. The mostly used failure criteria are the Von Mises (VM) overall/equivalent stress [18–22,26–28], Tresca (T) maximum shear stress [2,10–12], maximum principal S1 tensile stress [19,22,29–32], minimum principal S3 compressive stress [22,29,30,32–35], and hydrostatic pressure (HP) [36–40]. However, a recurrent issue in these studies (except in ours [2,10–12]) is the lack of correlations between the inner anatomical micro-structure, the material type resemblance, the criteria suitability, the coherent biomechanical behavior resembling to clinical knowledge, the quantitative results correlated with the physiological maximum hydrostatic pressure (MHP), the orthodontic force dissipation and absorption ability, and the biomechanically correct stress display. Thus, FEA is still approached with care since results are often supplied that contradict clinical knowledge [2,10–12].

In the engineering field, the FEA simulations are extremely accurate since all the above issues related to diverse types of correlations are addressed and the adequate failure criteria and correct input data have been defined. To have the same accuracy of the FEA method in dental studies, it is necessary that a single failure criterion addressing all above correlations is assessed to be scientifically accurate and providing results correlated with clinical and theoretical knowledge [2,10–12]. Previous studies from our group reported the ductile resemblance and showed that only VM and T criteria met all the above expectations, with Tresca proven to be more accurate for the tooth structural components, PDL, and dental pulp with NVB [2,10–12]. Thus, the bone FEA study herein completes the data necessary for assessing the general failure criteria for the tooth and surrounding support periodontium.

The objectives of this FEA analysis are (a) to assess the biomechanical behavior of mandibular bone subjected to light orthodontic forces during a horizontal periodontal breakdown; (b) to assess its suitability for the study in bone of five of the most used failure criteria employed in dental tissue research; (c) to correlate the results with other FEA-related reports of dental tissues to identify a suitable single unitary failure criteria for the study of teeth and the surrounding periodontium.

2. Materials and Methods

The numerical analysis herein is part of a larger stepwise research project (clinical protocol 158/02.04.2018) continuing the investigation of biomechanical behavior of teeth and surrounding periodontal structure during orthodontic movements and various levels of periodontal breakdown.

The earlier analyses of this project, with a focus on the dental pulp, neuro-vascular bundle (NVB), periodontal ligament (PDL), dentine and enamel, were conducted using the same models, boundary conditions, and physical properties as herein [2,10–12].

The 405 FEA numerical simulations were conducted over eighty-one 3D mandibular models holding the second lower premolar obtained from nine patients (4 males/5 females, mean age 29.81 $\pm$ 1.45). The selected convenience sample size of nine was acceptable for the accuracy of the results since most of the earlier FEA studies employed a sample size of one (one patient, one 3D model, and few simulations) [1,2,5,8,10–22,26–40]. The research project inclusion criteria were the presence of non-inflamed periodontium and various levels of bone loss, an intact arch and second premolar tooth structure, lack of endodontic treatment and malposition in the region of interest, indication of orthodontic treatment, and regular follow-up. All the situations that were not covered by the above criteria were considered to be exclusion criteria (especially the lack of arch integrity, tooth malposition, more than 8 mm bone-loss cases, and inflamed periodontium).

The lower mandibular region containing the premolars and first molar was examined by CBCT (ProMax 3DS, Planmeca, Helsinki, Finland), obtaining images of various shades of gray, with a voxel size of 0.075 mm.

The radiological Hounsfield gray shade units present on the DICOM slices were examined to identify the dental tissues. The anatomically accurate reconstruction of the tissues was performed through manual segmentation since the automated software algorithm did not accurately identify all the structures. Thus, the enamel, dentine, dental pulp, neurovascular bundle, periodontal ligament, cortical and trabecular bone were reconstructed in 3D (Figure 1). The reconstruction software was Amira 5.4.0 (Visage Imaging Inc., Andover, MA, USA). The base of the bracket, assumed to be of stainless steel, was reconstructed on the vestibular side of the enamel crown. Since the separation of the dentine and the cementum was impossible, and due to similar physical properties, the entire dentine–cementum structure was reconstructed as dentine (Table 1). The PDL had a variable thickness of 0.15–0.225 mm and included the NVB of the dental pulp in the apical third. The 3D models guarded only the second lower premolar, while the other tooth structures were replaced by cortical and trabecular bone. The missing bone and PDL (which were found in the cervical third) were reconstructed as closely as possible to the anatomical reality. Thus, nine models with intact periodontium (one from each patient) were obtained. In each of these models, a gradual horizontal breakdown process (0–8 mm of loss) was simulated by reducing both bone and PDL by 1 mm, obtaining a total of eighty-one models with various levels of bone loss. The 3D intact periodontium models had 5.06–6.05 million C3D4 tetrahedral elements, 0.97–1.07 million nodes, and a global element size of 0.08–0.116 mm.

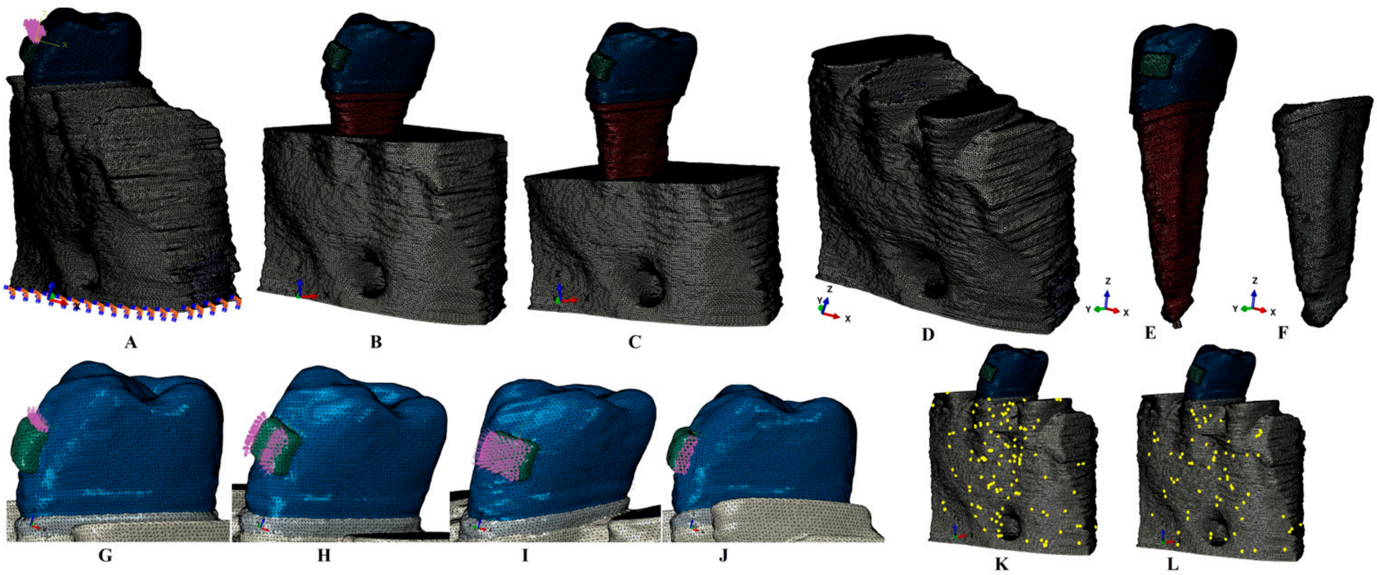

Figure 1. (**A**) 2nd lower right premolar model with intact periodontium, and applied vectors (encastered model base and extrusion loads); (**B**) 4 mm bone loss; (**C**) 8 mm bone loss; (**D**) bone structure (with cortical and trabecular components); (**E**) tooth model with bracket, enamel, dentin and neuro-vascular bundle, (**F**) intact PDL; applied vectors: (**G**) intrusion, (**H**) rotation, (**I**) tipping, (**J**) translation; (**K**) element warnings of the cortical bone component; (**L**) elements warnings of the cortical component.

The surface of the models, due to the manual segmentation technique, displayed a limited number of element warnings and no element errors (Figure 1). Thus, for one of the models shown in Figure 1K,L, the cortical bone mesh displayed 131 element warnings for 3,417,625 elements (i.e., 0.00383%), while for the trabecular mesh, there were only 70 element warnings for 1,699,730 elements (i.e., 0.00411%). The element warnings and