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Bacterial Biofilm Growth on Various Dental Stabilization Systems for Avulsed and Luxated Teeth

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Abstract: With the increased incidence of traumatic injuries and the advanced understanding of the periodontal and alveolar healing process, teeth splinting has become a common practice for stabilizing traumatized teeth. Consequently, several splinting materials and techniques have been introduced in the past few years. Despite the detrimental role of bacterial biofilm on healing, the level of biofilm development on these material surfaces has not been well investigated. Bacterial biofilms are severely detrimental for periodontal healing of avulsed and luxated teeth. Thus, biofilm growth becomes a critical factor in selecting the material of choice for dental splints. In this study, we aim to assess the level of oral biofilm growth on four different splinting systems: Ribbond©, orthodontic NiTi wire, monofilament fishing line, and Titanium Trauma Splint. A total of 72 extracted anterior teeth were divided into four groups. We splinted six rows of three teeth each per group. The teeth selected were caries-free and periodontitis-free at the time of extraction. To assess biofilm growth, a supragingival dental plaque sample was cultured and directly inoculated into all groups. After 7 days, bacterial growth was quantified by live/dead fluorescent microscopy assay and colony forming unit counts (CFU). Using one-way ANOVA and Bonferroni's post hoc tests, we demonstrated that all splint systems allowed for bacterial growth. However, the Titanium Trauma Splint (TTS) allowed for the least amount of biofilm growth compared to other splint systems.

Keywords: dental splint; trauma; bacterial biofilm; avulsion; luxation; dental; Titanium Trauma Splint

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1. Introduction

Traumatic injuries have become a common occurrence in today's society and may surpass the incidence of caries and periodontal disease [1]. Studies have confirmed that the prevalence of these injuries is increasing and ranges from 16% to 40% among 6-year-old children and from 4% to 33% among 12 to 14-year-old children [1,2]. A significant proportion of dental trauma relates to sports, unsafe playgrounds or schools, road accidents, or violence. Skaare and colleagues reported that in a group of children aged 7 to 10 years, the maxillary anterior teeth were the most frequently affected by trauma [3].

Generally, except for concussion and subluxation, these traumatic injuries require some type of stabilization for proper teeth retention and periodontal ligament healing [4–6]. Conventionally, several methods have been advocated for splinting such as fishing lines, orthodontic wires, orthodontic brackets, resins, and most recently introduced, the TTS system [5,6]. The TTS system is made of titanium and has been reported to be easy to use, significantly rapid to place and remove, and to facilitate proper hygiene [7,8].

It has been previously established that splints should be passive enough to allow for physiological healing; however, the cleanability of the splints has not been well studied [9–11]. One of the direct causes of inflammatory root resorption is bacterial contamination [12–15]. Pettini and Pettini have examined previously replanted teeth under

a scanning electron microscope and found a direct association between bacterial infection and resorptive defects [16]. Another study has demonstrated a tight association between inflammatory root resorption and periradicular bacterial infection in a mouse model observed by MicroCT [17].

Plaque level one of the paradoxes of dental splinting after trauma is that stabilizing the displaced tooth with a splint may lead to the accumulation of plaque, which hinders proper oral hygiene, causing bacterial contamination intra- and extraradicularly and causing root resorption [18]. Bacteria from the gingival sulcus have been detected at the root surface at the site of external resorption after traumatic injury [14,19]. In addition, periradicular bacteria may indirectly cause internal root resorption or pulpal necrosis [20]. A study by Grossman et al. concluded that an introduced nonoral bacteria to the oral cavity of monkeys with traumatized teeth found its way to the dental pulp [21]. Moreover, most traumatic injuries occur in children and adolescents who have larger dentinal tubules, allowing for the faster passage of oral bacteria [22,23]. With the advances in microbiological techniques, recent studies found a close resemblance of periodontal bacteria in the necrotic dental pulp after trauma [24,25]. Our new understanding of the importance of the bacterial load to the healing of the splinted avulsed or subluxated tooth emphasizes the importance of oral hygiene and reducing oral biofilm, especially during the first 2–3 weeks of treatment [18,26,27].

Furthermore, the International Association for Dental Traumatology recommends a splint that offers physiological retention and proper hygiene for a favorable clinical prognosis [28]. Previous studies have also reported that bacterial load and biofilm formation play a critical role in healing [8,29,30]. Yet, there has not been a study that investigated biofilm growth levels on the splint systems available on the market. In our study, we aim to compare biofilm growth on four different commonly used splinting systems currently being used by clinicians.

2. Materials and Methods

2.1. Teeth Selection and Bonding Process

Seventy-two caries- and periodontal disease-free maxillary anterior freshly extracted human teeth were used in this study. Teeth were extracted from patients at the University of Florida Oral Surgery department according to the patient's treatment plan. Teeth were collected according to the IRB protocol (201500591) and stored in saline solution. Teeth were sterilized using autoclave and then randomly divided into 4 different splint groups: monofilament fishing line (MFL), size 0.016 round NiTi orthodontic wire (OW) (Rocky Mountain Orthodontics®, Denver, CO, USA), 3-mm lock-stitch woven polyethylene ribbon (Ribbond®, Seattle, WA, USA), and TTS (Medartis Inc, Basel, Switzerland). Each group contained 6 replicates composed of 3 teeth each, in which the horizontal linear dimension of each group was approximately 28.0 mm. In addition, the splint length was set at 28 mm for all samples. Splints in each group were bonded with one drop (~0.05 g) of bonding agent (Scotchbond, 3M ESPE, St. Paul, MN, USA) and light-cured after etching with 35% phosphoric acid for 15 s. Then, 0.3 $\pm$ 0.01 g of flowable composite (Filtek Supreme Plus resin, 3M ESPE, St. Paul, MN, USA) attachments were consistently adapted to the labial surfaces of the extracted maxillary teeth following the manufacturer's specifications. Composite attachments were light-cured for 30 s following splint placement (Figure 1).

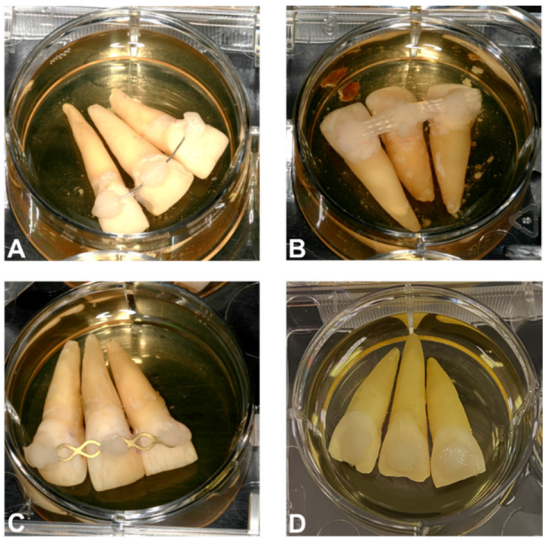

Figure 1. Teeth groups splinted with either (**A**) Orthodontic wire, (**B**) Ribbond, (**C**) Titanium Trauma Splint, or (**D**) Monofilament fishing line. Splinted teeth submerged completely in 3 mL of peptone yeast glucose (PYG) broth and replenished equally as needed.

2.2. Biofilm Preparation

A sterile curette was utilized to collect supragingival plaque samples from periodontal disease- and caries-free sites from properly consented individuals (IRB200600154). Samples were immediately placed into a 1-mL aliquot of Amies (1967) transport medium, supplemented with 0.5% gelatin (Fisher Scientific, Ocala, FL, USA) and 0.1% sodium thioglycollate (Fisher Scientific), and stored at 4 °C for 2 h or until further use. The collected dental plaque sample was then inoculated into 7 mL peptone yeast glucose (PYG) broth (Remel, San Diego, CA, USA) and allowed to grow for 24 h at 37 °C in a CO_2 incubator. This culture was then directly inoculated into each well of 6-well culture plates that contained 3 mL of fresh PYG broth (1:50 dilution). Splinted teeth were completely submerged in the broth (Figure 1). Samples were incubated with low-speed agitation in a CO_2 chamber at 37 °C for 7 days for biofilm growth. Media were replenished every 48 h.

2.3. Cell Viability Assay

On day 7, the samples were removed from the wells, and splints were carefully detached from the teeth. The samples were sonicated in 1 mL of Ringer's solution for 15 s. The sonicated samples were then serially diluted and plated onto blood agar plates, enriched with hemin and vitamin K, to assess for the number of viable cells present. The remaining solution was prepared for a live/dead fluorescence assay. From each sample, 100 µL was placed in triplicate in a 96-well black polystyrene microplate and mixed with 100 µL of live/dead dye (50 µL SYTO 9 working solution, 50 µL PI working solution) (Live/dead Backlight kit, Invitrogen, Carlsbad, CA, USA). The plate was then incubated in the dark at room temperature with gentle shaking for 20 min and absorbance was measured at Ex/Em 528/485 nm (live cells) and Ex/Em 645/485 nm (dead cells) using a Biotek plate reader (BioTek, Winooski, VT, USA). The live/dead ratio was calculated after subtracting blank (media only) and plotted into a previously built standard curve ($r^2 = 0.99$) to obtain the cell viability ratio. PYG broth incubated for 7 days was included as a control. The results were analyzed with a one-way ANOVA and data were plotted on a dot plot graph.

2.4. Statistical Analysis

All data were checked for normality using Shapiro–Wilk's test. CFUs and live/dead ratios were compared among groups by ANOVA with Bonferroni's multiple comparison post hoc test.

3. Results

Differences in bacterial growth were observed comparing all splinting systems. Fluorescence intensity was used to measure the ratio of live/dead cells present in each group (Figure 2). We found that TTS resulted in a mean of 0.0505 ± 0.007 (95% CI, 0.03184–0.06916), while MFL, Ribbond, and OW resulted in 1.275 ± 0.062 (95% CI, 1.117–1.439), 1.583 ± 0.1053 (95% CI, 1.239–1.780), and 1.470 ± 0.0584 (95% CI, 1.307–1.607), respectively ($p < 0.0001$). TTS showed a 25×, 31×, 29× reduction in viable cells compared to MFL, Ribbond, and OW, respectively ($p < 0.0001$), with no differences found among the other three splint systems (Figure 2).

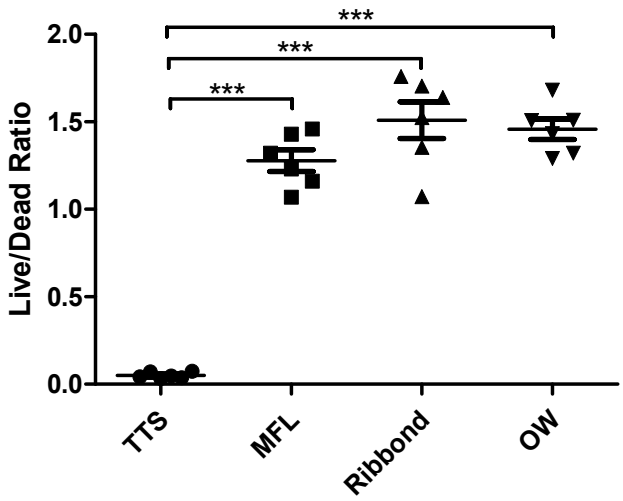

Figure 2. Live/dead ratio plot for the groups tested. Each group is presented by mean and standard error horizontal lines. NiTi orthodontic wire (OW), woven polyethylene ribbon (Ribbond®), Titanium Trauma Splint (TTS), and monofilament fishing line (MFL). *** $p < 0.0001$.

Further, a CFU assay was performed to determine the number of viable cells in the culture after 7 days (Figure 3). We found that the mean number of viable cells in TTS was 4.567×10^6, while MFL, Ribbond, and OW had a viable count of 4.467×10^{10}, 5.400×10^{10}, and 4.400×10^{10}, respectively ($p = 0.0047$). The resultant colony count concluded that TTS had a significantly lower number of viable bacterial cells than any other splint system tested after 7 days of incubation ($p = 0.0047$), with no differences found among the other three splint systems.

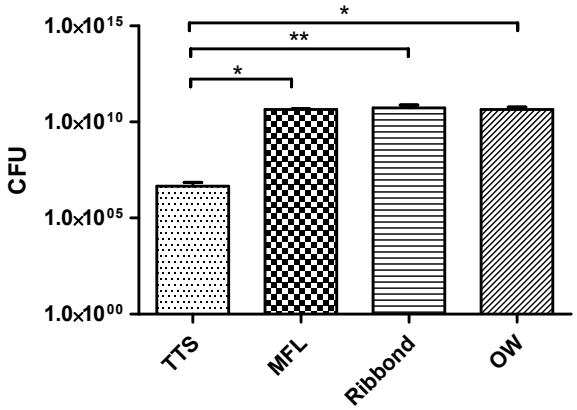

Figure 3. Colony forming unit values (CFUs) showing the number of viable cells in each sample. MFL: monofilament fishing line, OW: NiTi orthodontic wire, Ribbond: lock-stitch woven polyethylene ribbon, TTS: Titanium Trauma Splint. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Bacterial biofilm is not a static community as it is a continuously growing and striving biosystem [31,32]. Biofilm composition and virulence level increase with age and the overall growth of the biofilm [33,34]. As the biofilm matures and bacterial load increases, there is a higher chance for bacteria to expand into the lateral canals and dentinal tubules, and bacteria found at these sites are reported to become more resistant to antibiotics [35,36]. Thus, it is critical to select a splint material that allows the least amount of biofilm growth in addition to the physiological stabilization ability. Furthermore, traumatic injuries are commonly associated with soft tissue injuries and lacerations, which are prone to bacterial contamination. Thus, the presence of bacterial biofilm on the splint system and the surrounding teeth exacerbates the inflammation of the surrounding tissues and contributes to delayed healing.

In addition, bacterial root resorption is the main complication causing the majority of failures detected following trauma [29,37]. Currently, according to the International Dental Traumatology guidelines for Traumatic Injuries, with the exception of alveolar fractures, the splint should be physiologic (non-rigid) for the duration of two weeks for avulsion and luxation injuries, and 6 to 8 weeks for mid-root horizontal root fractures [4,38].

One of the materials we tested is the monofilament nylon line, "fishing line". It was introduced in 1982, and has been used with success by some practitioners [39]. Additionally, nylon monofilament has been used as a suture material in several types of surgeries. It has been shown to allow for some but limited biofilm growth compared to other suture materials, especially multifilament ones [40]. Currently, new splint systems have been introduced into the market such as Rebbond [41,42], OW [43], and TTS [7,8], and while practitioners differ in their choice, the common agreement is that they should be easy to apply and remove, be esthetic, and facilitate hygiene. However, bacterial growth on these newer systems has not been properly evaluated before.

Since the presence of bacteria is so detrimental for the healing of post-traumatic injuries, this study focused on determining which type of splint system would favor the least amount of biofilm growth. When splints were bonded with the same amount of composite to the labial surfaces of three maxillary teeth, they all allowed bacterial growth. However, we observed significantly less bacterial growth in TTS when compared to the other three materials, MFL, Ribbond, and OW, which showed comparable bacterial growth to one another. Multiple factors may play a role in the level of bacterial adherence and growth, such as surface roughness, surface area, and splint design. Surface roughness is a critical factor for biofilm adherence to the splint surface [44] or surface treatment that may facilitate bacterial adherence [45,46]. In addition, the architectural design of the splint system may also play a role in allowing for biofilm detachment. We have shown for the first time that the new TTS system allows for the least amount of biofilm retention in vitro. Our study was limited to the quantification of the bacterial growth on each splint. Further investigation into the types of bacteria collected would provide us with a better understanding if the splint material can select for certain bacterial types. In addition, future clinical studies measuring biofilm adherence and growth in the oral cavity and cleanability by patients would also be desirable.

5. Conclusions

Several splint systems are available on the market to facilitate the physiological splinting of avulsed and luxated teeth. While all materials allowed for bacterial biofilm formation, TTS has been shown to be the least promoting for biofilm formation and thus a more attractive splint system for clinicians.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Petti, S.; Glendor, U.; Andersson, L. World traumatic dental injury prevalence and incidence, a meta-analysis-One billion living people have had traumatic dental injuries. *Dent. Traumatol.* **2018**, *34*, 71–86. [CrossRef]
2. Artun, J.; Behbehani, F.; Al-Jame, B.; Kerosuo, H. Incisor trauma in an adolescent Arab population: Prevalence, severity, and occlusal risk factors. *Am. J. Orthod. Dentofacial. Orthop.* **2005**, *128*, 347–352. [CrossRef]
3. Skaare, A.B.; Jacobsen, I. Dental injuries in Norwegians aged 7–18 years. *Dent. Traumatol.* **2003**, *19*, 67–71. [CrossRef]
4. Flores, M.T.; Malmgren, B.; Andersson, L.; Andreasen, J.O.; Bakland, L.K.; Barnett, F.; Bourguignon, C.; DiAngelis, A.; Hicks, L.; Sigurdsson, A.; et al. Guidelines for the management of traumatic dental injuries. III. Primary teeth. *Dent. Traumatol.* **2007**, *23*, 196–202. [CrossRef] [PubMed]
5. Flores, M.T.; Andersson, L.; Andreasen, J.O.; Bakland, L.K.; Malmgren, B.; Barnett, F.; Bourguignon, C.; DiAngelis, A.; Hicks, L.; Sigurdsson, A.; et al. Guidelines for the management of traumatic dental injuries. II. Avulsion of permanent teeth. *Dent. Traumatol.* **2007**, *23*, 130–136. [CrossRef]
6. Flores, M.T.; Andersson, L.; Andreasen, J.O.; Bakland, L.K.; Malmgren, B.; Barnett, F.; Bourguignon, C.; DiAngelis, A.; Hicks, L.; Sigurdsson, A.; et al. Guidelines for the management of traumatic dental injuries. I. Fractures and luxations of permanent teeth. *Dent. Traumatol.* **2007**, *23*, 66–71. [CrossRef] [PubMed]
7. von Arx, T.; Filippi, A.; Lussi, A. Comparison of a new dental trauma splint device (TTS) with three commonly used splinting techniques. *Dent. Traumatol.* **2001**, *17*, 266–274. [CrossRef]
8. von Arx, T.; Filippi, A.; Buser, D. Splinting of traumatized teeth with a new device: TTS (Titanium Trauma Splint). *Dent. Traumatol.* **2001**, *17*, 180–184. [CrossRef] [PubMed]
9. Kristerson, L.; Andreasen, J.O. The effect of splinting upon periodontal and pulpal healing after autotransplantation of mature and immature permanent incisors in monkeys. *Int. J. Oral Surg.* **1983**, *12*, 239–249. [CrossRef]
10. Andreasen, J.O. Treatment of fractured and avulsed teeth. *ASDC J. Dent. Child* **1971**, *38*, 29–31.
11. Nasjleti, C.E.; Castelli, W.A.; Caffesse, R.G. The effects of different splinting times on replantation of teeth in monkeys. *Oral Surg. Oral Med. Oral Pathol.* **1982**, *53*, 557–566. [CrossRef]
12. Abbott, P.V. Prevention and management of external inflammatory resorption following trauma to teeth. *Aust. Dent. J.* **2016**, *61* (Suppl. 1), 82–94. [CrossRef] [PubMed]
13. Fuss, Z.; Tsesis, I.; Lin, S. Root resorption–diagnosis, classification and treatment choices based on stimulation factors. *Dent. Traumatol.* **2003**, *19*, 175–182. [CrossRef]
14. Trope, M. Luxation injuries and external root resorption-etiology, treatment, and prognosis. *J. Calif. Dent. Assoc.* **2000**, *28*, 860–866.
15. Paula-Silva, F.W.G.; Ribeiro-Santos, F.R.; Petean, I.B.F.; Manfrin Arnez, M.F.; Almeida-Junior, L.A.; Carvalho, F.K.; Silva, L.A.B.D.; Faccioli, L.H. Root canal contamination or exposure to lipopolysaccharide differentially modulate prostaglandin E 2 and leukotriene B 4 signaling in apical periodontitis. *J. Appl. Oral Sci.* **2020**, *28*, e20190699. [CrossRef]
16. Pettini, F.; Pettini, P. Root resorption of replanted teeth: An SEM study. *Endod. Dent. Traumatol.* **1998**, *14*, 144–149. [CrossRef]
17. Balto, K.; White, R.; Mueller, R.; Stashenko, P. A mouse model of inflammatory root resorption induced by pulpal infection. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2002**, *93*, 461–468. [CrossRef]
18. Fouad, A.F. Microbiological Aspects of Traumatic Injuries. *J. Endod.* **2019**, *45*, S39–S48. [CrossRef]
19. Al-Nazhan, S.A.; Spangberg, L.W. Light and SEM observation of internal root resorption of a traumatized permanent central incisor. *Int. Endod. J.* **1995**, *28*, 133–136. [CrossRef]
20. Galler, K.M.; Gratz, E.M.; Widbiller, M.; Buchalla, W.; Knuttel, H. Pathophysiological mechanisms of root resorption after dental trauma: A systematic scoping review. *BMC Oral Health* **2021**, *21*, 163. [CrossRef]
21. Grossman, L.I. Origin of microorganisms in traumatized, pulpless, sound teeth. *J. Dent. Res.* **1967**, *46*, 551–553. [CrossRef]
22. Love, R.M. The effect of tissue molecules on bacterial invasion of dentine. *Oral Microbiol. Immunol.* **2002**, *17*, 32–37. [CrossRef]
23. Love, R.M.; Jenkinson, H.F. Invasion of dentinal tubules by oral bacteria. *Crit. Rev. Oral Biol. Med.* **2002**, *13*, 171–183. [CrossRef]

24. Gomes, B.P.; Berber, V.B.; Kokaras, A.S.; Chen, T.; Paster, B.J. Microbiomes of Endodontic-Periodontal Lesions before and after Chemomechanical Preparation. *J. Endod.* **2015**, *41*, 1975–1984. [CrossRef]
25. Rupf, S.; Kannengiesser, S.; Merte, K.; Pfister, W.; Sigusch, B.; Eschrich, K. Comparison of profiles of key periodontal pathogens in periodontium and endodontium. *Endod. Dent. Traumatol.* **2000**, *16*, 269–275. [CrossRef] [PubMed]
26. Andreasen, F.M.; Kahler, B. Pulpal response after acute dental injury in the permanent dentition: Clinical implications—A review. *J. Endod.* **2015**, *41*, 299–308. [CrossRef]
27. Andreasen, J.O.; Lauridsen, E.; Andreasen, F.M. Contradictions in the treatment of traumatic dental injuries and ways to proceed in dental trauma research. *Dent. Traumatol.* **2010**, *26*, 16–22. [CrossRef]
28. Andersson, L.; Andreasen, J.O.; Day, P.; Heithersay, G.; Trope, M.; Diangelis, A.J.; Kenny, D.J.; Sigurdsson, A.; Bourguignon, C.; Flores, M.T.; et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dent. Traumatol.* **2012**, *28*, 88–96. [CrossRef]
29. Andreasen, J.O.; Vinding, T.R.; Christensen, S.S.A. Predictors for healing complications in the permanent dentition after dental trauma. *Endod. Top.* **2006**, *1*, 20–27. [CrossRef]
30. Andreasen, J.O.; Jensen, S.S.; Sae-Lim, V. The role of antibiotics in preventing healing complications after traumatic dental injuries: A literature review. *Endod. Top.* **2006**, *14*, 80–92. [CrossRef]
31. Yawata, Y.; Nguyen, J.; Stocker, R.; Rusconi, R. Microfluidic Studies of Biofilm Formation in Dynamic Environments. *J. Bacteriol.* **2016**, *198*, 2589–2595. [CrossRef]
32. Fagerlind, M.G.; Webb, J.S.; Barraud, N.; McDougald, D.; Jansson, A.; Nilsson, P.; Harlén, M.; Kjelleberg, S.; Rice, S.A. Dynamic modelling of cell death during biofilm development. *J. Theor. Biol.* **2012**, *295*, 23–36. [CrossRef]
33. Alves, F.R.; Siqueira, J.F.; Carmo, F.L.; Santos, A.L.; Peixoto, R.S.; Rôças, I.N.; Rosado, A.S. Bacterial community profiling of cryogenically ground samples from the apical and coronal root segments of teeth with apical periodontitis. *J. Endod.* **2009**, *35*, 486–492. [CrossRef]
34. Siqueira, J.F.; Rôças, I.N. Community as the unit of pathogenicity: An emerging concept as to the microbial pathogenesis of apical periodontitis. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2009**, *107*, 870–878. [CrossRef]
35. Ricucci, D.; Siqueira, J.F. Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. *J. Endod.* **2010**, *36*, 1–15. [CrossRef]
36. Ricucci, D.; Loghin, S.; Siqueira, J.F. Exuberant Biofilm infection in a lateral canal as the cause of short-term endodontic treatment failure: Report of a case. *J. Endod.* **2013**, *39*, 712–718. [CrossRef]
37. Bastos, J.V.; Côrtes, M.I.S. Pulp canal obliteration after traumatic injuries in permanent teeth-scientific fact or fiction? *Braz. Oral Res.* **2018**, *32*, e75. [CrossRef] [PubMed]
38. Fouad, A.F.; Abbott, P.V.; Tsilingaridis, G.; Cohenca, N.; Lauridsen, E.; Bourguignon, C.; O'Connell, A.; Flores, M.T.; Day, P.F.; Hicks, L.; et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dent. Traumatol.* **2020**, *36*, 331–342. [CrossRef] [PubMed]
39. Antrim, D.D.; Ostrowski, J.S. A functional splint for traumatized teeth. *J. Endod.* **1982**, *8*, 328–331. [CrossRef]
40. Bucci, M.; Borgonovo, A.; Bianchi, A.; Zanellato, A.; Re, D. Microbiological analysis of bacterial plaque on three different threads in oral surgery. *Minerva. Stomatol.* **2017**, *66*, 28–34. [CrossRef]
41. Chaudhary, V.; Shrivastava, B.; Bhatia, H.P.; Aggarwal, A.; Singh, A.K.; Gupta, N. Multifunctional Ribbond–a versatile tool. *J. Clin. Pediatr. Dent.* **2012**, *36*, 325–328. [CrossRef] [PubMed]
42. Bansal, R.; Chowdhary, P.; Gurtu, A.; Mehrotra, N.; Kishore, A. Splinting of Longitudinal Fracture: An Innovative Approach. *Case Rep. Dent.* **2016**, *2016*, 5083874. [CrossRef] [PubMed]
43. Oikarinen, K. Tooth splinting: A review of the literature and consideration of the versatility of a wire-composite splint. *Endod. Dent. Traumatol.* **1990**, *6*, 237–250. [CrossRef]
44. Taha, M.; El-Fallal, A.; Degla, H. In vitro and in vivo biofilm adhesion to esthetic coated arch wires and its correlation with surface roughness. *Angle Orthod* **2016**, *86*, 285–291. [CrossRef] [PubMed]
45. Yoshinari, M.; Oda, Y.; Kato, T.; Okuda, K. Influence of surface modifications to titanium on antibacterial activity in vitro. *Biomaterials* **2001**, *22*, 2043–2048. [CrossRef]
46. Chouirfa, H.; Bouloussa, H.; Migonney, V.; Falentin-Daudré, C. Review of titanium surface modification techniques and coatings for antibacterial applications. *Acta Biomater.* **2019**, *83*, 37–54. [CrossRef]