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# Dentistry & Otolaryngology

Highlights

- Recent Host Modulation Therapy
- Palate after Uranoplasty in Children
- Different Extracts of Celtis Australis
- Secondary and Residual Deformities

**Discovering Thoughts, Inventing Future**

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# The State of the Local Cytokine Status and its Pathogenetic Significance with Secondary and Residual Deformities of the Palate after Uranoplasty in Children

By D. M. Dusmukhamedov, A. A. Khadzhimetov, Z.K. Khakimova & D. K. Dusmukhamedova

*The Relevance of the Research-* The provision of qualified care to patients with congenital cleft of the upper lip and palate (CCLP), accompanied by dentoalveolar anomalies and nasal deformities is one of the most difficult tasks of modern dentistry and maxillofacial surgery . According to various authors, complications after reconstructive operations range from 8 to 32% (4,5,8,13,14). In this pathology, the quality of the postoperative scar depends on the general condition of the body, the nature of the disease, the experience of the surgeon, the type of suture material and many other factors. Any surgical intervention in the dento-maxillary system causes disturbances in microcirculation, as well as blood circulation of tissues around the wound, which leads to an inflammatory reaction. Even with the initial wound healing, accompanied by a decrease in blood supply, the scar forms and matures more slowly, and its quality is worse. The interest in the problem of postoperative wound healing is explained by the fact that inflammation plays a leading role in the course of any wound process, which determines the path along which wound healing will go.

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# The State of the Local Cytokine Status and its Pathogenetic Significance with Secondary and Residual Deformities of the Palate after Uranoplasty in Children

D. M. Dusmukhamedov [α], A. A. Khadzhimetov [σ], Z.K. Khakimova [ρ] & D. K. Dusmukhamedova [ω]

## I. The Relevance of the Research

The provision of qualified care to patients with congenital cleft of the upper lip and palate (CCLP), accompanied by dentoalveolar anomalies and nasal deformities is one of the most difficult tasks of modern dentistry and maxillofacial surgery . According to various authors, complications after reconstructive operations range from 8 to 32% (4,5,8,13,14). In this pathology, the quality of the postoperative scar depends on the general condition of the body, the nature of the disease, the experience of the surgeon, the type of suture material and many other factors. Any surgical intervention in the dento-maxillary system causes disturbances in microcirculation, as well as blood circulation of tissues around the wound, which leads to an inflammatory reaction. Even with the initial wound healing, accompanied by a decrease in blood supply, the scar forms and matures more slowly, and its quality is worse. The interest in the problem of postoperative wound healing is explained by the fact that inflammation plays a leading role in the course of any wound process, which determines the path along which wound healing will go. Considering the medical and social significance of the problem of healing postoperative wounds in the tissues of the maxillofacial area, the development of methods aimed at optimizing the healing process of postoperative wounds, reducing the number of complications and improving the appearance of scars remains an urgent problem in surgical dentistry. Recently, it is proved that the factors affecting wound healing, and cell interaction is the normal work of cells and cytokines. Consequently, the regeneration of tissues in the oral cavity depends on adequate cellular cooperation. Growth factors play an important role in the development of scars. Growth factors are polypeptides that release various activated cells at the site of injury. They stimulate cell proliferation and chemoattraction of new cells. The variety of clinical manifestations n after conducting various s kinds and techniques uranoplasty, in particular arising from the secondary (SD) (post-operative) and residual defects (RD) of the sky in children, as well as difficulties in treating them do to date and the need for further study of their pathogenesis and improve the s methods of treatment.

*The aim of our study was-* to evaluate with local cytokine status and its pathogenic role in secondary and residual defects of the palate after uranoplasty children.

## II. Material and Research Methods

To clarify the frequency, localization and mechanisms of development of secondary and residual palate defects in connection with the use of various uranoplasty techniques, we studied 47 archival case histories of children with CCLP who were treated in the department of pediatric surgical dentistry of the Andijan regional hospital in the period from 2010-2019. and pediatric maxillo - facial surgery clinic of the Tashkent State Dental Institute in the period 2010-2019 gg. To systematize residual and secondary defects and deformities of the upper lip, alveolar ridge and palate, the classification of E.N. Samara (1977, 1981), where the author identifies the following forms: defects of hard, hard and soft, soft, connected defects. In terms of size, defects can be: small (up to 1 cm), medium (up to 2 cm), large (more than 2 cm).

As you know, the results of uranoplasty largely depend on the completeness of the restoration of the anatomy of the palate and on the correct position of the pathologically altered muscles of the soft palate, which provide the palatopharyngeal closure. Our retrospective analysis of the case histories of patients with secondary (SD) and residual defects (RD) of the palate in children with CCLP shows that they have a peculiar clinical picture. The clinical picture of RD and SD of the palate after uranoplasty largely depends on the shape of the cleft and the method of uranoplasty, while the SD and RD of the palate have the most common favorite localizations: they were located along the former cleft, had a different shape and size - from 3 to 22 mm. The most common complications of uranoplasty is the discrepancy of the sutures (RD) at the border of the hard and soft palate 18.5%. RDs of this localization, as a

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rule, develop due to the anatomical features of the cleft and technical errors of the operation. The results of a retrospective analysis of case histories showed that 41 (87.2 %) patients in the preoperative period had a severe somatic background - as prescribed by the pediatrician, they received antianemic treatment for several months, often received anti-inflammatory drug therapy and were somewhat lagging behind in physical development from their peers. Consequently, secondary and residual defects, as well as de formation of the sky, are often the result of a defective examination and treatment of patients in the preoperative and postoperative periods. To study the state of local immunity in children with secondary and residual palatal deformities after uranoplasty, we selected patients after diagnosis, depending on the result of primary uranoplasty, and were divided into the following groups: group 1 (n = ...) consisted of children without local complications after uranoplasty; Group 2 (n = ...) - children with RD and SD of the palate after uranoplasty and group 3 (n = ...) - comparison group, children without pathology of the dentition. All studies were conducted with informed consent. The cytokines IL-1, IL-6, IL-8, TNF-a, and TGF-R were determined by enzyme immunoassay using “HUMAN” kits. Cytokines IL-1, IL-6, TNF-a, belonging to the group of pre-immune inflammation or primary pro-inflammatory cytokines. Secondary proinflammatory cytokines include chemokines, a large group of more than 50 proteins. In our study, this group is represented by IL-8. Anti-inflammatory cytokines: TGF- R. For the work, we used statistical methods of descriptive statistics, correlation analysis, establishing the reliability of the difference between data in the main and control groups on the basis of calculating the Student's test. Data in the text and tables are given as M ± m (mean value ± standard error of its mean). Results with a significance level of $<0.05$ (95% confidence interval) were considered reliable.

## III. Research Results and their Discussion

As it is known, in any phase of the surgical interverence possibly a protracted course of healing of the wound process, with sluggish growth of granulation and delayed epithelization. Slowing down of wound healing occurs with a decrease in immunity indicators, for example, caused by a prolonged increase in the level of steroid hormones. The use of glucocorticoids (GCs) in the early postoperative period causes a significant decrease in the number and functions of immunocompetent cells, inhibition of angiogenesis, fibroblast proliferation, and synthesis of components of the extracellular matrix. In this situation, HA reduces the normal expression of proinflammatory cytokines, which is required for wound healing. The mechanism of action of glucocorticoids is inhibition of the transcription of certain genes, or in the suppression of the activation of NF -KB and. Glucocorticoids inhibit the synthesis of proinflammatory cytokines, in particular IL -1, as well as the expression of the growth factors TGF - P and their receptors, which is reflected in the slowing down of the maturation of granulation tissue, which induces the synthesis of KGF in fibroblasts. Tumor necrosis factor (TNF - a), produced by macrophages, is a pro-inflammatory cytokine and plays a role in collagen synthesis. All this leads to reduction reepitelization wounds.

Considering that children with secondary and residual defects and deformations of the sky after uranoplasty in this area marked activation of a range of immunological mechanisms aimed at preventing the generalization of the pathologic process, we studied the local and general n itokinovy profile in this group of children with the purpose of determining their values in its flow. Informative in our opinion, is the study of cytokines in oral fluid and serum, which allows the system to evaluate the reaction of the organism in the presence of a pathological process in the oral tissues.

*Table 1:* Cytokine profile of blood serum and oral fluid in children with secondary and residual defects and deformities of the palate after uranoplasty

| Indicator | I- group( n = 16) | II- group( n = 22) | III- group( n = 24 ) |
|---|---|---|---|
| Bloodserum | | | |
| IL-1 , pg / ml | 6.85 ± 0.54 | 8.81 ± 0.61 | 5.29 ± 0.38 |
| IL-6, pg / ml | 5.34 ± 0.41 | 9.87 ± 0.72 | 4.05 ± 0.31 |
| IL-8, pg / ml | 2.60 ± 0.24 | 6.28 ± 0.53 | 1.74 ± 0.13 |
| TNF-a , pg / ml | 2.45 ± 0.22 | 20.99 ± 1.28 | 1.89 ± 0.15 |
| TFR-r, pg / ml | 4.01 ± 0.26 | 4.96 ± 0.35 | 3.71 ± 0.26 |

| Oralfluid | | | |
|---|---|---|---|
| IL-1 , pg / ml | 120.05 ± 9.62 | 25.12 ± 2.52 | 139.72 ± 20.05 |
| IL-6, pg / ml | 55.86 ± 4.03 | 22.59 ± 7.93 | 41.58 ± 3.69 |
| IL-8, pg / ml | 47.55 ± 3.31 | 16.94 ± 9.98 | 39.87 ± 3.53 |
| TNF-a , pg / ml | 2.02 ± 6.84 | 0.29 ± 6.07 | 1.54 ± 0.11 |
| TFR-r, pg / ml | 2.82 ± 0.36 | 1.54 ± 0.37 | 3.68 ± 0.27 |

*Note: * - reliability of differences P < 0, 05 relative to the comparison group*

As can be seen from the presented research results (Table 1), as a result of a decrease in the microbial load in the examined children, changes in the cytokine profile of blood serum occur, which are difficult to interpret, but from the point of view of their functional significance, IL-1,6, 8, TNF- a, that is, all pro-inflammatory cytokines, as well as TGF-R, which is necessary for the induction of regeneration processes, activation of fibroblasts - cells that are producers of collagen, elastin, proteoglycans. At the same time, TGF-R promotes the growth of blood vessels during reparative regeneration. With regard to the immune response in general, TGF-R manifests itself as an immunosuppressive agent. The importance of TGF-R is confirmed by the fact that it is one of three cytokines that is always detected in blood serum. Perhaps this is due to the fact that the processes of cell death and their restoration are always parallel in the body.

Interestingly, the concentration of IL-1R was significantly lower in the oral fluid in children with defects. In POSSIBILITY, this is due to the depletion of the cytokine in connection with long-flowing chronic inflammatory process. This assumption is indirectly confirmed by the fact that the use of antimicrobial therapy, due to which the microbial load decreases and, consequently, the inflammatory potential decreases, does not significantly increase the level of IL-1R, but, on the contrary, decreases it. The explanation for the findings of the study is that Porphyromonas gingivalis leads to a decrease in the production of IL-1R (3). It is known that IL- 10 is a potent inhibitor of macrophages and their antigen-presenting function, and also inhibits the production of cytokines of active T-lymphocytes, namely, they synthesize TGF-R, one of the main participants in regeneration. It turned out that the level of serum TNF was significantly increased, while in the oral fluid it was significantly reduced. TNF participates in the formation of a focus of local inflammation, creating barriers that can preserve the localization of the pathogen, and also induces the synthesis of IL-1 and IL-6, the main participants in the full response of the acute phase, which is necessary for the adequate course of all stages of inflammation and their full regeneration.

Presented studies indicate that in the oral fluid and blood serum of children surveyed come multidirectional changes in the concentration pro-inflammatorycytokines and growth factors. Thus, there is a clear relationship between systemic cytokine pro Lemma and the process of healing wounds in children with secondary and residual defects of palate and strains after uranoplasty. The results of studies with one hand indicate values cytokines straight and wound healing that is of great interest of researchers , on the other of the SIC causes reduction epithelialization and dividing of wound healing and reduce e reparative processes in children with secondary and residual Defects and deformities of the palate after uranoplasty. Revealing the facts apparently due to a decrease m the production of IL -1 in the wound surface on a background of the use of glucocorticoids in early post operative period. Consequently, it can be concluded that disfunction production of cytokines, particularly IL-1 at the wound surface is one of the reasons of complicated wound healing in children with secondary and residual Defects sky and strains after uranoplasty.

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# Protective Effect of Different Extracts of Celtis Australis and Syzygium Aromaticum on Tetrahymena for the Cytotoxicity of Nickel-Titanium-Based Orthodontic Wires

By Nassiba Fatene, Mar Papa Daouda, Rachida Cadi, Abdelaziz Soukri & Khadija Mounaji

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*Abstract-* *Introduction:* The present work aims to study the toxicity of orthodontic archwires based on Nickel-Titanium and to evaluate the protective effect of different types of extracts of *Celtis australis* and *Syzygium aromaticum*, considered as natural corrosion inhibitors, on *Tetrahymena thermophila* and *Tetrahymena pyriformis*.

*Methods:* *Tetrahymena thermophila* and *Tetrahymena pyriformis* were cultured in artificial saliva previously incubated in the presence of NiTi or CuNiTi wires with or without the addition of different types of plant extracts (extract, hydrosols, essential oils). The effect of wires and plant extracts on *Tetrahymena* was evaluated after 2, 4 and 7 days of growth, by the protozoan viability test and the microscopic observation of the shape.

*Results:* For the two *Tetrahymena* species, NiTi and CuNiTi cause a decrease in *Tetrahymena* growth by about 50%.

*Keywords:* *orthodontic wires, nickel-titanium, cytotoxicity, aromatic plants, tetrahymena.*

*GJMR-J Classification:* *NLMC Code: WU 426*

*Strictly as per the compliance and regulations of:*

# Protective Effect of Different Extracts of Celtis Australis and Syzygium Aromaticum on Tetrahymena for the Cytotoxicity of Nickel-Titanium-Based Orthodontic Wires

Nassiba Fatene [α], Mar Papa Daouda [σ], Rachida Cadi [ρ], Abdelaziz Soukri [ω] & Khadija Mounaji [¥]

*Abstract- Introduction:* The present work aims to study the toxicity of orthodontic archwires based on Nickel-Titanium and to evaluate the protective effect of different types of extracts of *Celtis australis* and *Syzygium aromaticum*, considered as natural corrosion inhibitors, on *Tetrahymena thermophila* and *Tetrahymena pyriformis*.

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*Results:* For the two *Tetrahymena* species, NiTi and CuNiTi cause a decrease in *Tetrahymena* growth by about 50%. The extract of *Celtisaustralis* added to NiTi or CuNiTi, shows a protective effect on the growth of the protozoan. In contrast, essential oils have no protective effect against NiTi or CuNiTi.

*Conclusions: Celtis australis* extract could be considered a protective agent for *Tetrahymena* against the cytotoxic effect of nickel-titanium-based orthodontic wires.

*Keywords: orthodontic wires, nickel-titanium, cytotoxicity, aromatic plants, tetrahymena.*

## I. Introduction

The biocompatibility of orthodontic materials has been widely studied due to the importance of this property for patient safety. Most orthodontic materials contain metals, which can be toxic and produce allergic reactions[1,2]. It has been proven that in the oral environment, orthodontic archwires undergo chemical corrosion leading to the release of ions in saliva[3]. Nickel-Titanium wires are an important part of the therapeutic arsenal during fixed orthodontic treatment. They contain about 47-50% of Nickel and are the richest source of this metal in the oral cavity of most patients with orthodontic appliances. Furthermore, Nickel and Titanium are known for their toxic and carcinogenic effects[4-6].

In the literature, cell culture is the most widely used method to assess the toxicity of orthodontic materials in the oral environment. Several other models for studying toxicity were described. Among them, *Saccharomyces cerevisiae* have been used to study orthodontic material cytotoxicity[7]. Other microorganisms have also been used in toxicology, like the ciliated protozoan *Tetrahymena*[8].

Unlike other single-cell microorganisms that are widely used as models, this protozoan has the advantage of having several genes found in several eukaryotes, including humans[9]. More than 800 human genes have orthologs in *Tetrahymena thermophila*, but not in *S. cerevisiae*, 58 of them are associated with human diseases[10]. This characteristic suggests that *Tetrahymena* can be used as a model to improve the understanding of the molecular mechanisms involved in the toxicity of orthodontic materials[11].

On the other hand, to stop alloy'scorrosion, researchers tested several methods. Among them, the use of natural corrosion inhibitors or green corrosion inhibitors extracted from aromatic plants has been widely studied in industry[12]. Indeed, the use of different types of aromatic plant extracts (essential oils, hydrosols and extracts) has a protective effect against the corrosion of metals in an acid environment avoiding by the way the use of chemical substances[13]. In addition, it has been described that some aromatic plants, such as *Artemisia* and *Syzygium aromaticum*, have anti-corrosive properties[14-16].

The aim of this work is to assess the cytotoxicity of Nickel-Titanium-based orthodontic archwires and to study the protective effect of different types of aromatic plant extracts, considered as natural corrosion

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inhibitors, using *Tetrahymena thermophila* and *Tetrahymena pyriformis* as study models.

## II. Material and Methods

*a) Culture of Tetrahymena*

*Tetrahymena thermophila* SB 1969 and *Tetrahymena pyriformis* SE, ATCC30005 were used for this study. Both species were kept growing in the PPYE medium containing 0.5% (w/v) of Proteose Peptone and 0.2% (w/v) of yeast extract. Artificial saliva was prepared by adding to the PPYE medium 0.035% (w/v) of Sodium Chloride (NaCl), 0.2% (w/v) of Calcium Chloride (CaCl2) and 0.2% (w/v) Potassium Chloride (KCl). Then, in this culture medium was added 1% (v/v) of a pre-culture of *Tetrahymena thermophila* (1.5×105 cells/ml) and incubated at 32°C. or of *Tetrahymena pyriformis* (104 cells/ml) and incubated at 28°C. In order to check the growth and adaptation of the protozoan to artificial saliva, pre-cultures were carried out and monitored for 3 months. Then, during one year, a transplanting was carried out once a week.

*b) Preparation of wires and plant extracts*

NiTi (3M) and CuNiTi (ORMODENT, California) orthodontic arch-wires were cut into 10mm pieces and then sterilized. The different types of extracts were prepared from *Syzygium aromaticum* (Clove) and *Celtis australis*. The essential oil and the hydrosol were obtained by hydrodistillation using a Clevenger type device (2 liter reactor), for a period of five hours. These extracts were then stored inamber glass bottles at a temperature of 4°C.

The extract was obtained by macerating the powder of the leaves of *Celtis australis* in distilled water-methanol (2V/3V) for 48 hours at 25°C.

The essential oil and hydrosol of *Syzygium aromaticum*, the extract and the essential oil of *Celtis australis* were chosen for this study (the choice of plant extracts and concentrations used was based on the results obtained by our team; results being published).

*c) Assessment of the effect of orthodontic archwires and the anti-corrosion potential of different types of plant extracts on the growth of Tetrahymena*

Each piece of orthodontic archwire was incubated in 20ml of artificial saliva with or without the addition of the extract, hydrosol or essential oils, as shown in detail in Figure 1. These media were incubated at 37°C for 15 days with agitation to simulate the oral conditions. Then, these media were distributed in 4 tubes, of 5 ml each, then inoculated with a pre-culture of *Tetrahymena thermophila* (1.5x105 cells/ml) or *Tetrahymena pyriformis* (104 cells/ml).

Protozoan growth was monitored during 7 days of culture by measuring the optical density at 600 nm using the spectrophotometer.

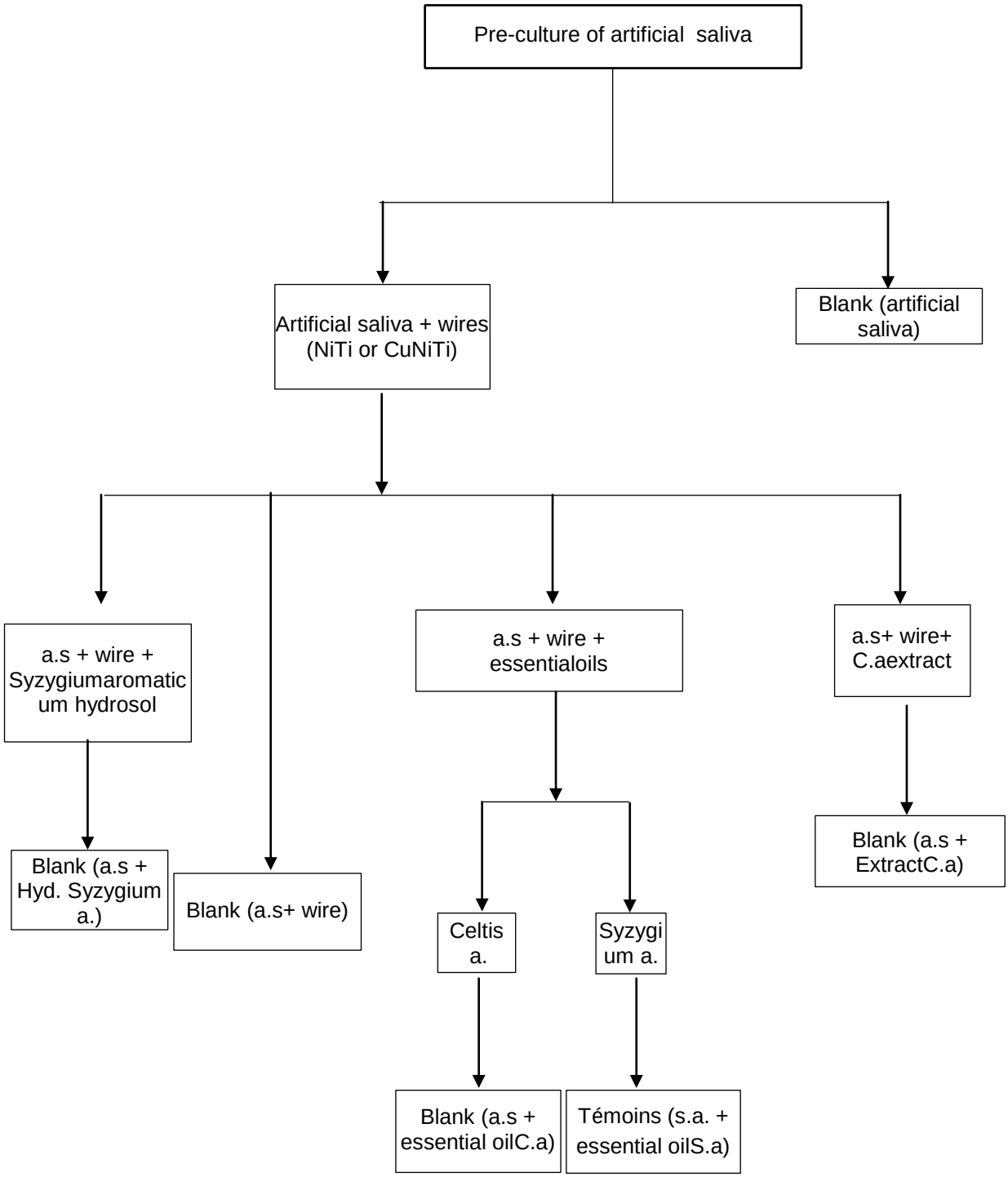


*Figure 1:* Distribution of different solutions and negative and positive controls. s.a: artificial saliva, NiTi: Nickel-Titanium, CuNiTi: Copper-Nickel-Titanium, S.a: Syzygium aromaticum, C.a: Celtis australis, Hyd: hydrosol.

*d) Evaluation of cell viability and morphology of Tetrahymena*

In order to calculate the percentage of living cells and to analyse the shape of the protozoan, a sample of 20 µl of each culture medium was taken after 48 h, 96 h and 169 h of growth of *Tetrahymena*. These samples were stained with Trypan blue (2%), fixed with Formaldehyde (4%) and then placed in a Malassez cell for observation under the microscope.

*e) Statistical analysis*

Three replicates were made for each experiment and the mean and standard deviation were calculated. Statistical analysis was performed using Student's T-test and the differences were considered statistically significant if $p<0.05$.

## III. Results

*a) Growth of Tetrahymena thermophila and Tetrahymena pyriformis in artificial saliva*

Results show that in artificial saliva, the growth curves of *Tetrahymena thermophila* and *Tetrahymena pyriformis* are not modified in comparison with the PPYE medium (Figure 2).

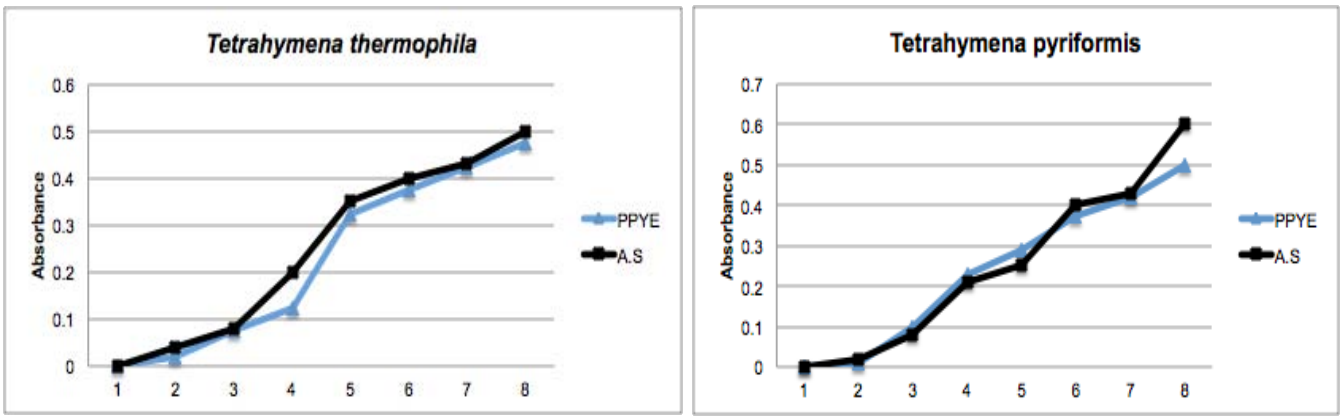


*Figure 2:* Growth kinetics of Tetrahymena thermophila and Tetrahymena pyriformis in artificial saliva and PPYE medium during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva

Results of the viability analysis in the two *Tetrahymena* species after 48h, 96h and 168h of culture do not show any significant differences between the artificial saliva and the PPYE medium (Figure 3).

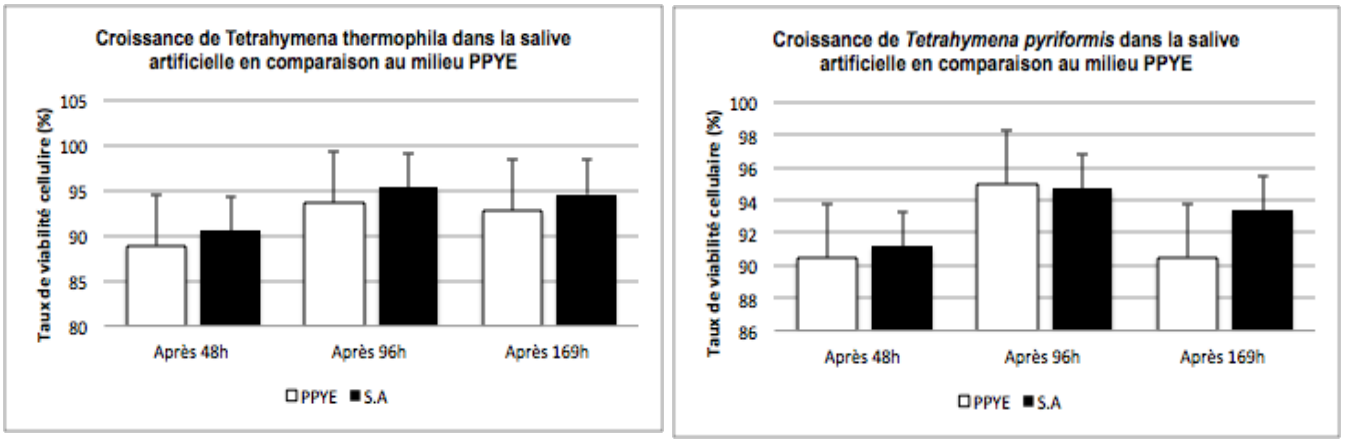


*Figure 3:* Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in artificial saliva (S.A) and the PPYE medium. There is no statistically significant difference between the two environments.

Similarly, observation under the microscope does not show any change in the shape of the two species of *Tetrahymena* in artificial saliva compared to the PPYE medium (Figure 4).

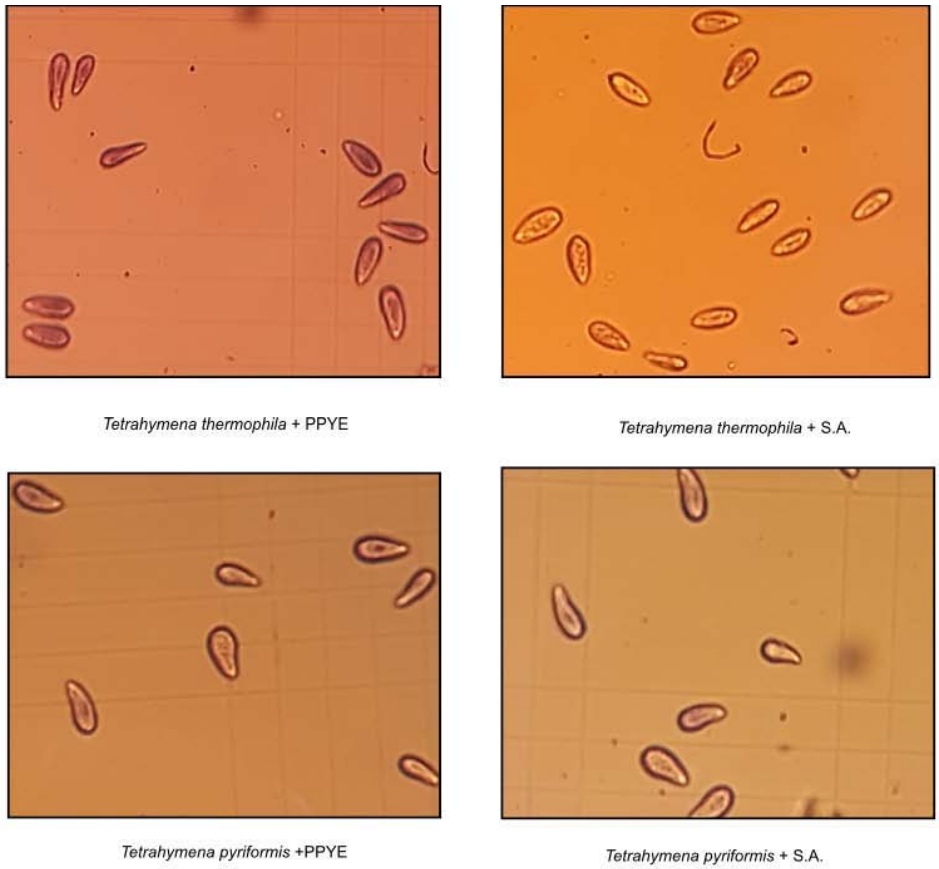


*Figure 4:* Microscopic images of Tetrahmyena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 100 magnification, showing the comparison of growth in artificial saliva and the usual medium PPYE

*b) Cytotoxic effect of orthodontic archwires on Tetrahymena*

In the presence of NiTi or CuNiTi orthodontic archwires, the growth of *Tetrahymena thermophila* is significantly reduced by 50% and 60% respectively compared to controls (Artificial saliva) ($p < 0.01$) (Figure 5).

The same results are noted in *Tetrahymena pyriformis* the growth of *Tetrahymena pyriformis* is reduced by 72% and 60% respectively compared to the controls (artificial saliva) ($p < 0.01$) (Figure 5).Results of the morphology analysis show that in the presence of orthodontic archwires, the majority of the protozoan appears in 2 shapes; elongated and rounded with a blue color after Trypan blue test compared to control (Figure 6).

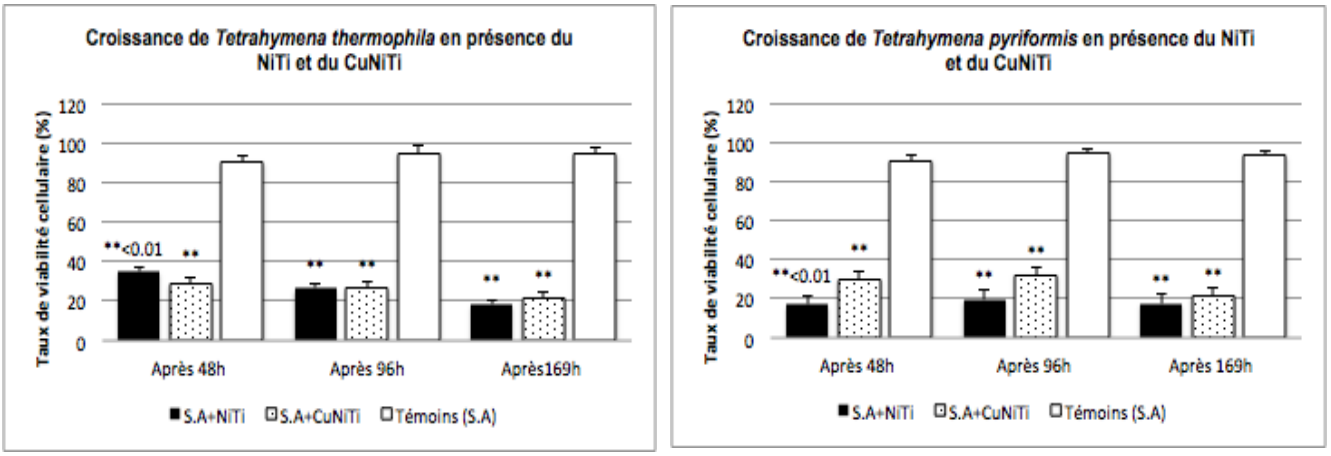


*Figure 5:* Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of the NiTi and CuNiTi arcs alone. The differences are statistically significant ($p < 0.01$).
S.A: artificial saliva

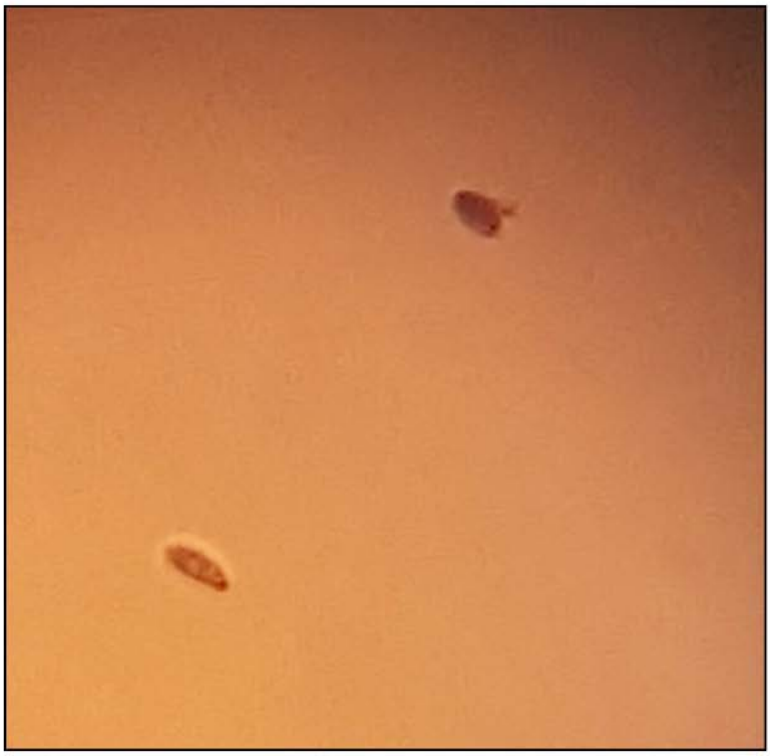

*Figure 6:* Rounded or elongated forms of Tetrahymena in the presence of orthodontic archwires objectifying their cytotoxicity

c) *Effect of the different types of extracts of Syzygium aromaticum and Celtis australis on the cytotoxicity of the wires*

The protozoan was cultured in artificial saliva previously incubated, for 15 days, in the presence of NiTi or CuNiTi wires and the different types of extracts of *Syzygium aromaticum* and *Celtis australis*.

i. *Effect of Celtis australis extracts on the growth of Tetrahymena*

During the protozoan growth kinetics, the number of living cells was counted during the 3 essential phases of the normal growth cycle of *Tetrahymena*: latency phase (24h), exponential phase (72h) and stationary phase (168h).

When the protozoan was cultured in the presence of the extract of *Celtis australis* and NiTi or CuNiTi, a remarkable increase in the number of living cells was noted for the two species compared to control (artificial saliva+arch)(figure 7). This increase was about 40% during the first phase of protozoan growth and the growth continues to increase during the second and third phase. The growth curves of *Tetrahymena thermophila* and *Tetrahymena pyriformis* almost align with those of control (artificial saliva alone). However, *Celtis australis* essential oil did not show any protective effect on the growth of *Tetraymena* in the presence of wires (Figure 7).

Extract of *Celtis australis* protects the two species of *Tetrahymena against the effect of* NiTi and CuNiTi; the majority of cells presents a pear shape, which characterizes the normal shape of the protozoan, at the end of the latency phase (figure 8).

In solutions containing the extract of *Celtis australis* alone, there is no statistically significant difference in the growth of *Tetrahymena thermophila* and *Tetrahymena pyriformis* compared to the control (artificial saliva alone) (Figure 7).

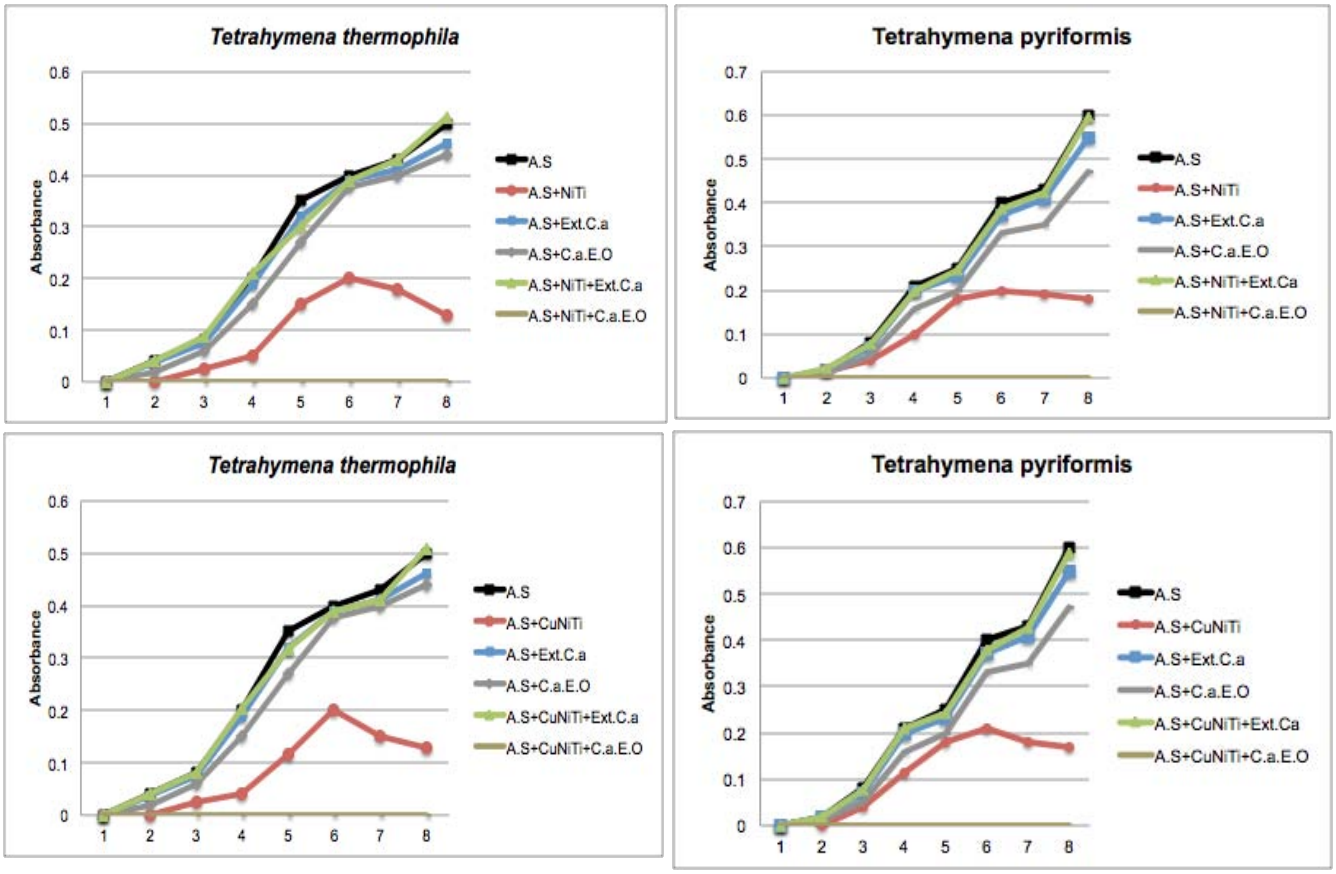


*Figure 7:* Effect of different types of Celtis australis extracts on the cytotoxicity of NiTi and CuNiTi arcs on Tetrahymena during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva, NiTi: Nickel Titanium, CuNiTi: copper Nickel Titanium, Ext.C.a: Celtis australis extract, C.a.E.O: Celtis australis essential oil.

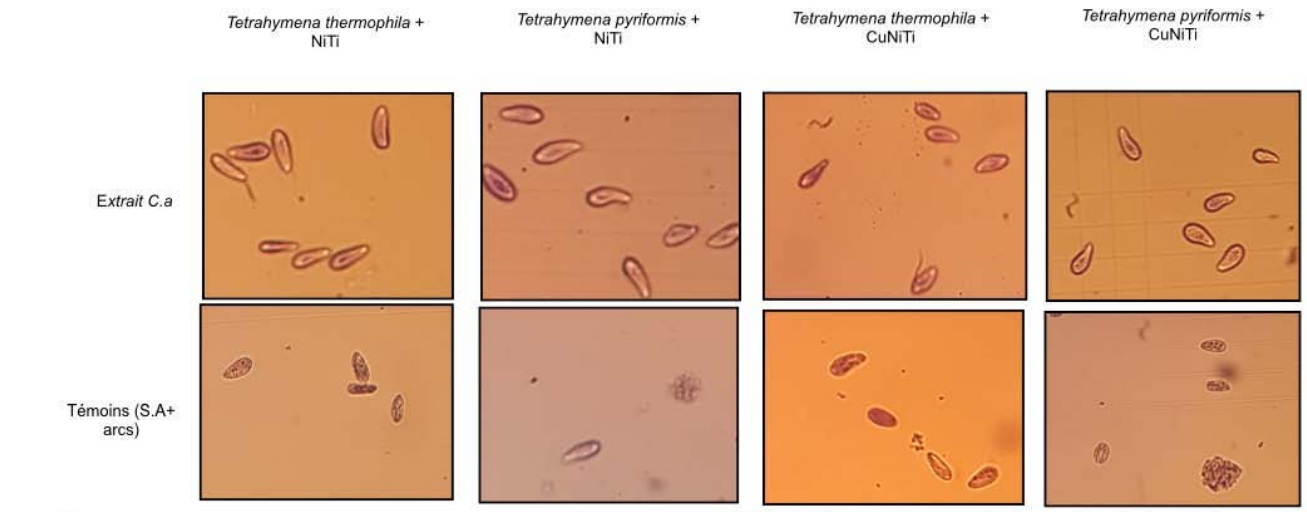


*Figure 8:* Microscopic images of Tetrahymena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 100 magnification, showing the comparison of their form in artificial saliva and the presence of NiTi and CuNiTi + the extract of Celtis autralis.
C.a: Celis australis, S.A: artificial saliva.

ii. *Effect of Syzygium aromaticum extracts on the growth of Tetrahymena*

In the presence of *Syzygium aromaticum* hydrosol alone with *Tetrahymena*, growth is approximately 80% ($p < 0.05$). Also, in the presence of the essential oil of *Syzygium aromaticum* alone, the growth is around 70% ($p < 0.05$).

In the solutions containing the wires and the hydrosol of *Syzygium aromaticum*, there is an increase in the rate of living cells by 50% during the 1st phase of

growth compared to the control ($p<0.05$). This growth decreases during the second (-20%) and the third (-60%) phase for the two *Tetrahymena* species. In addition, no growth was noted in the presence of the essential oil of *Syzygium aromaticum* for the two wires (Figure 8). All the differences are statistically significant except for the hydrosol of *Syzygium aromaticum* at the end of protozoan growth (Figure 9).

Regarding the morphology, in the solutions containing the hydrosol of *Syzygium aromaticum*, the two species of *Tetrahymena* show a pear shape at the end of the first phase of growth. In addition, from the second phase, the shape becomes rounded and the number and the mobility of cells decrease (Figure 10).

Figures 11 and 12 resume all the viability tests of *Tetrahyemna* in the presence of NiTi and CuNiTi with the different extracts of the two plants in comparison to those in the presence of plants extracts alone.

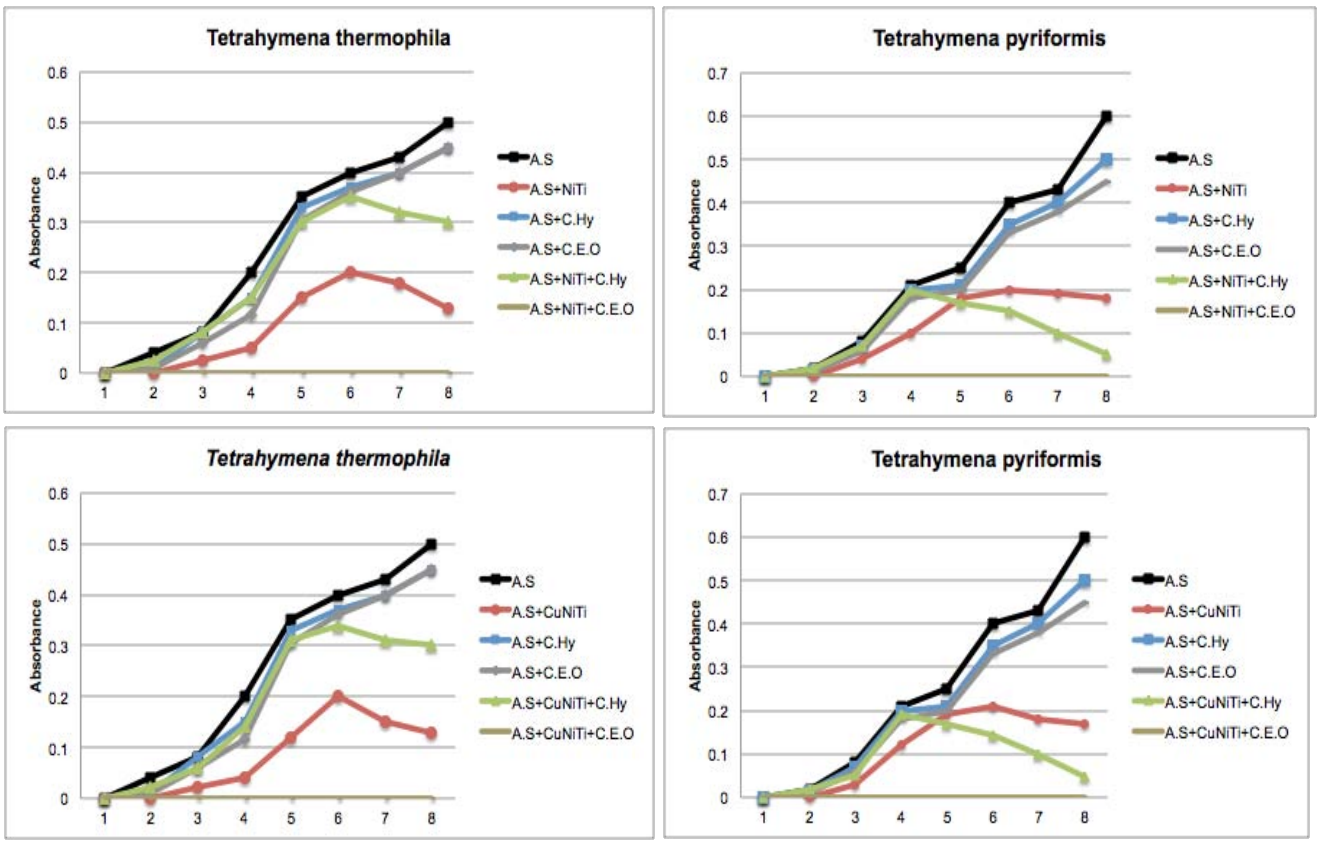


*Figure 9:* Effect of different types of Syzygium aromaticum (clove) extracts on the cytotoxicity of NiTi and CuNiTi arcs on Tetrahymena during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva, CuNiTi: copper Nickel Titanium, C.Hy: Clove hydrosol, C.E.O: Clove essential oil.

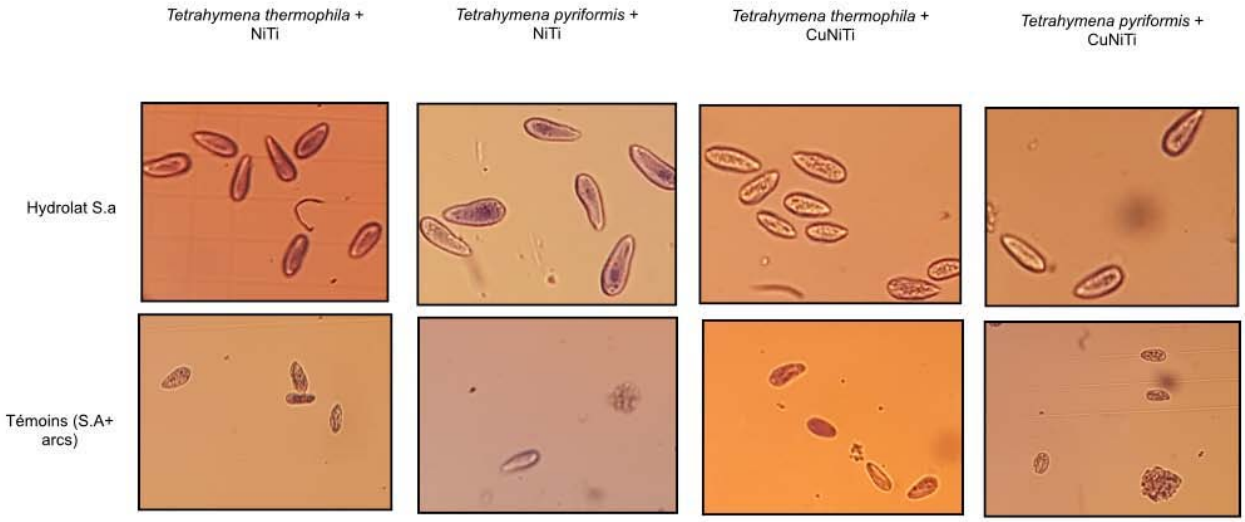


*Figure 10:* Microscopic images of Tetrahymena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 10 magnification, showing the comparison of their form in artificial saliva and the presence of NiTi and CuNiTi $^{+}$ the hydrosol of Syzygium aromaticum.
S.a: Syzygium, S.A: artificial saliva.

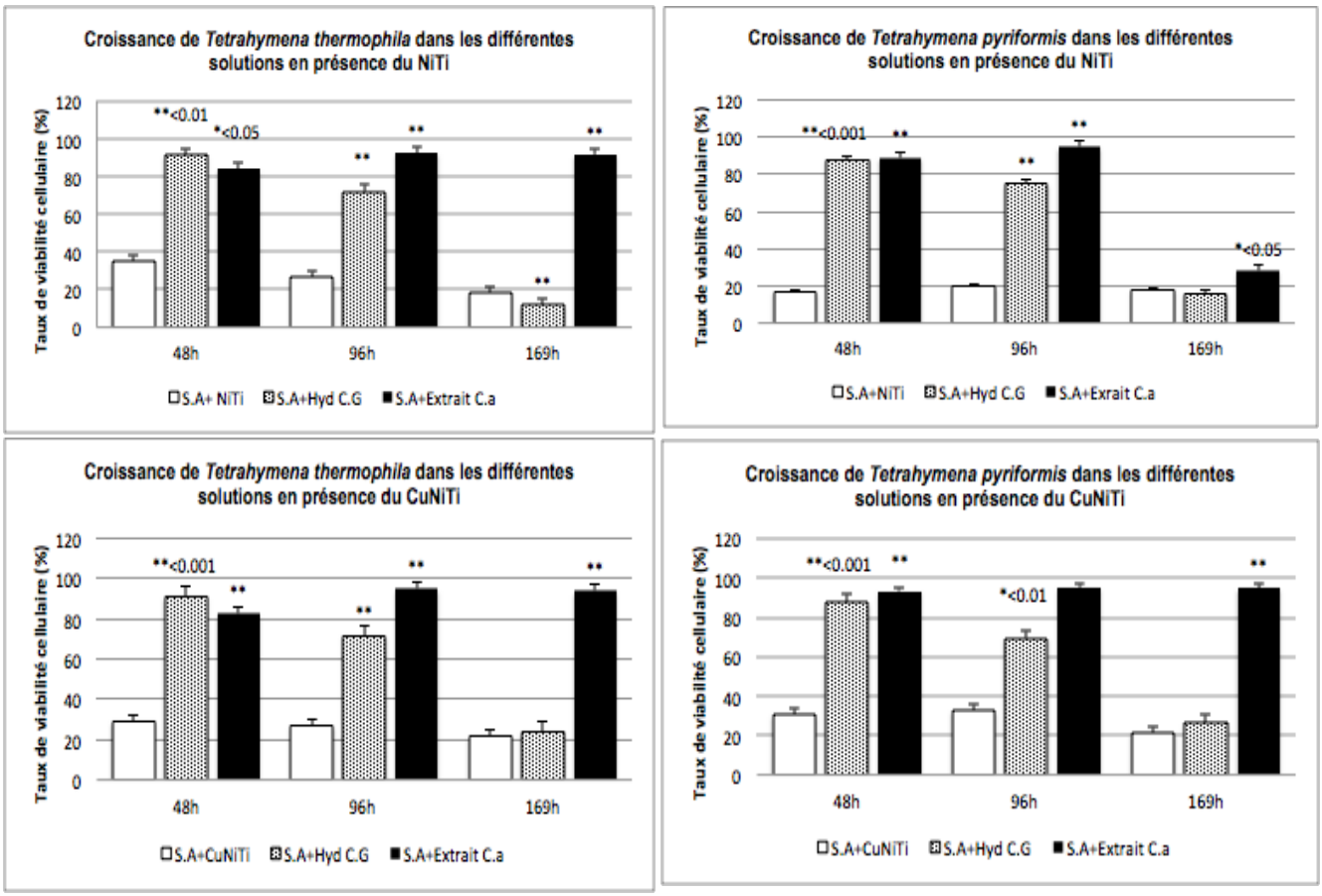


*Figure 11:* Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of NiTi and CuNiTi arcs and various natural corrosion inhibitors. The differences are statistically significant if $p < 0.05$, very significant if $p < 0.01$ and very very significant if $p < 0.001$.
S.A: artificial saliva, Hyd C; G: Hydrosol of cloves, Extract C.a: Extract of Celtis australis

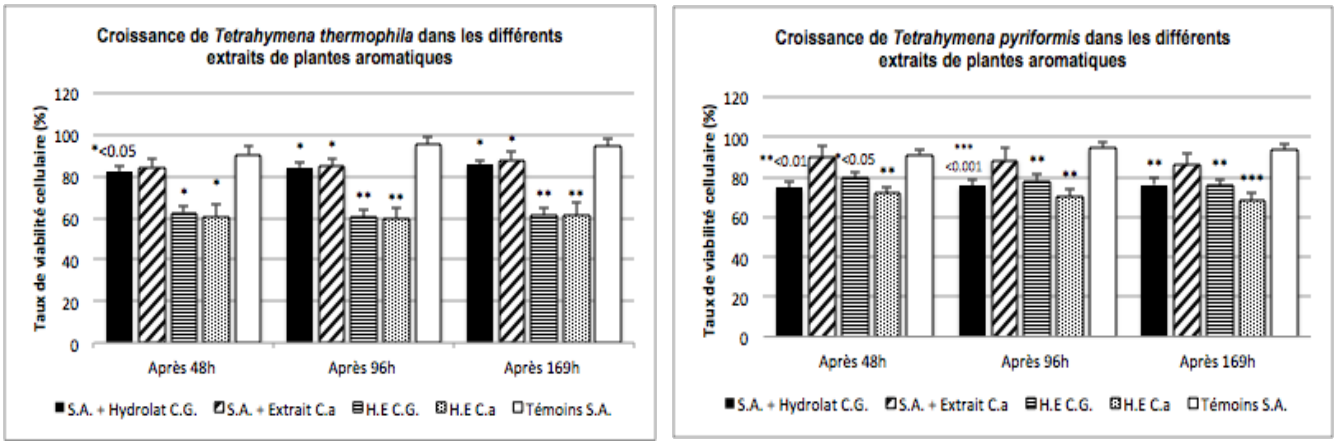


*Figure 12:* Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of the extract, essential oils and the hydrosol alone. The differences are statistically significant if $p < 0.05$, very significant if $p < 0.01$ and very very significant if $p < 0.001$.
S.A: artificial saliva, C.G: clove, C.a: Celtis australis, H.E: essential oil.

## IV. Discussion

Fixed orthodontic appliances must guarantee absolute safety and biocompatibility[17]. These qualities are of paramount importance in the oral cavity because this one constitutes a hostile chemical microenvironment that requires a high mechanical resistance of orthodontic alloys[18]. In the presence of saliva that acts as an electrolyte, the orthodontic archwires undergo corrosion that causes the release of metal ions in the environment[19]. To combat this corrosion, certain aromatic plants have proven their effectiveness as inhibitors of alloy corrosion[12].

The aim of this study was to assess the toxicity of Nickel-Titanium-based orthodontic archwires and to study the protective effect of different types of aromatic plant extracts, using *Tetrahymena* as a study model. Indeed, this protozoan constitutes a choice model for studies of environmental and industrial pollutants and of toxicity[20] and several studies has shown that *Tetrahymena* can constitute a reliable and effective biomarker for the estimation of toxic effects from several chemical wastes[21, 22].

In addition, studies have reported that this unicellular organism has similar genes to those of humans[10] and that it may also be useful in understanding the molecular mechanisms of toxicity in humans[23], this was the reason of its use in our study.

The perfect medium for *Tetrahymena*'s growth is PPYE; a medium that contains all the nutrients that the protozoan needs for its growth[24]. The use of this medium for toxicity tests of orthodontic archwires was not appropriate due to the absence of the elements constituting natural saliva. For this, our choice fell on artificial saliva; a culture medium which has already been described in the literature and adapted to the growth of *Tetrahymena*[25]. During one year, several pre-cultures of *Tetrahymena* were carried out, using artificial saliva, to have a generation perfectly adapted to this environment thus eliminating the specific stress due to artificial saliva. Our results have shown that the protozoan growth kinetic in artificial saliva is similar to the one of the PPYE medium.

In artificial saliva, *Tetrahymena* was cultured in the presence of NiTi or CuNiTi orthodontic wires to assess their cytotoxicity and the results showed a decrease in protozoan growth as well as a change in shape (elongated or rounded shape). Our results agree with those of Zhang and al.[26] who also showed a decrease in protozoan growth in the presence of heavy metals.

In addition, other work has reported that the released nickel and copper ions penetrate inside *Tetrahymena* and stop its growth[27, 28]. On the other hand, the released ions cause an unbalance between oxidants and antioxidants in the cell, inducing an oxidative stress that is involved in inflammation and in tumor pathology[29].

Our results showed that there is a protective effect of the extracts of *Celtis australis* and the hydrosol of *Syzygium aromaticum* against the toxicity of orthodontic archwires on *Terahymena*. Nilsson reported that the protozoan tolerates copper and nickel better in an organic solution than in a culture medium containing no nutrient[30, 31] which may explain the protective effect of the two extracts on the protozoan. Other authors have confirmed the protective effect of these two aromatic plants, which is consistent with our results[32-34]. These two plants are also known for their anticorrosive effect on metals that could have an indirect protective action on the protozoan by limiting the release of free radicals in the environment[35, 36]. Indeed, in a later study conducted by our team[37], a high corrosion of NiTi and CuNiTi wires under the same conditions as the present study was noted.

The effect of aromatic plants on *Tetrahymena* has been the subject of several works in our laboratory[38-40] and the protective effect of several essential oils (argan oil, sage and oregano) has been proven. However, the effect of essential oils and their corresponding extracts and hydrosols has never been studied on *Tetrhaymena*. The chemical composition of the extract and the hydrosol differs considerably from the corresponding essential oil [41], they contain a good concentration of the main molecule of the plant without the toxic phenolic substances constituting the essential oils[42].The results of this study show that the essential oil of *Syzygium aromaticum* and *Celtis autralis* have no protective effect on *Tetrahymena* against the cytotoxicity of orthodontic archwires by indirect action causing chemical corrosion which would increase the rate of ions present in saliva. On the other hand, the use of the extract of *Celtis australis* and the hydrosol of *Syzygium aromaticum* would protect the protozoan against the cytotoxicity of ions released in saliva.

## V. Conclusion

This study has shown that *Tetrahymena thermophila* and *Tetrahymena pyriformis* can constitute a model for studying the cytotoxicity of orthodontic materials. These cell cultures are simple to carry out, reproducible and inexpensive. In addition, the extract of *Celtis australis* could constitute a protective compound against the cytotoxicity generated by the corrosion of orthodontic archwires.

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# Comprehensive Diagnosis of an Invaginated Tooth Prior to Endodontic Treatment – A Clinical Case

By Igor Noenko & Volodymyr Fedak

*Abstract-* The article expores currently available ways of differential diagnosis of external and internal resorption in the presence of a related developmental abnormality, dens invaginatus (DI), to the maximum extent possible; whereas DI genuine etiology is still open to debate.

In different regions, the DI prevalence varies to a considerable extent. The non-occurrences are attributed to flawed diagnosis; therefore, not all DI cases are included in the statistics.

Meanwhile, such an invagination may lead to complications developing in the pulp and periapical tissues, and thereby it may significantly impede endodontic treatment.

*Objective:* The current study aims to study the intricacies of the dens invagination (DI) abnormality in routine dental practice. An attempt has been made to better understand the clinical signs of invagination and their impact on complications, and to systematize the criteria for diagnosing this abnomality.

*Keywords:* *etiology, dens invaginatus, dens in dente, classification.*

*GJMR-J Classification:* *NLMC Code: WU 230*


*Strictly as per the compliance and regulations of:*

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## I. Introduction

Dens invaginatus, or dens in dente, is a tooth maldevelopment with bizarre dental hard tissue arrangement due to the enamel organ invasion into the tooth pulp chamber before the dental tissues have become mineralized. It begins at the crown and sometimes extends into the root with formation of a pocket or dead space, or it is an accentuation of the lingual pit of an incisor before calcification sets in (Hegde et al.) Dens invaginatus is a rarther frequent malformation (2—3 %) (Grahnen et al., 1953).

The clinical case below illustrates the importance of comprehensive diagnosis in determining the tactics of endodontic treatment and revealing the cause of the endopathology.

Female patient K., 23 years old, was referred by an orthodontist. Orthodontic treatment was being planned and it was necessary to come up with the tactics of managing tooth 22. The following diagnostic tools were used:

1. Periapical X-rays;
2. Cone beam CT scans
3. Instrumental diagnostics was also employed, of which the cold test turned out to be the most informative.

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The X-ray snapshots showed signs of internal resorption in tooth 2.2.

The CBCT revealed intraroot perforating resorption on the vestibular root surface. In addition, a possible cause of resorption was identified as Oehlers' Type I invagination (1957), which was based on the radiological findings. According to the classification, Type I invagination is covered with enamel and is located within the coronal part, extending no further than the enamel-dentin junction. The authors believe that the infected invagination zone with subsequent creeping infection of the root pulp brought about the resorption. The response to the cold stimulus was very insignificant, especially in comparison with tooth 12. This made it clear that an irreversible destructive process is going on in the damaged tooth. Since the patient was planning orthodontic treatment and the resorption process could grow worse, it was decided to conduct endodontic treatment.

The diagnosis presented some difficulties and it was necessary to discriminate between internal and external resorption, as they require dififerent treatment tactics. While external resorption provides for either observation or surgery, depending on the extent of the defect and location, internal resorption often implies endodontic treatment.

The criteria for differential diagnosis included the following:

The radiographic findings were very similar to external resorption, but some moments were not typical of it.

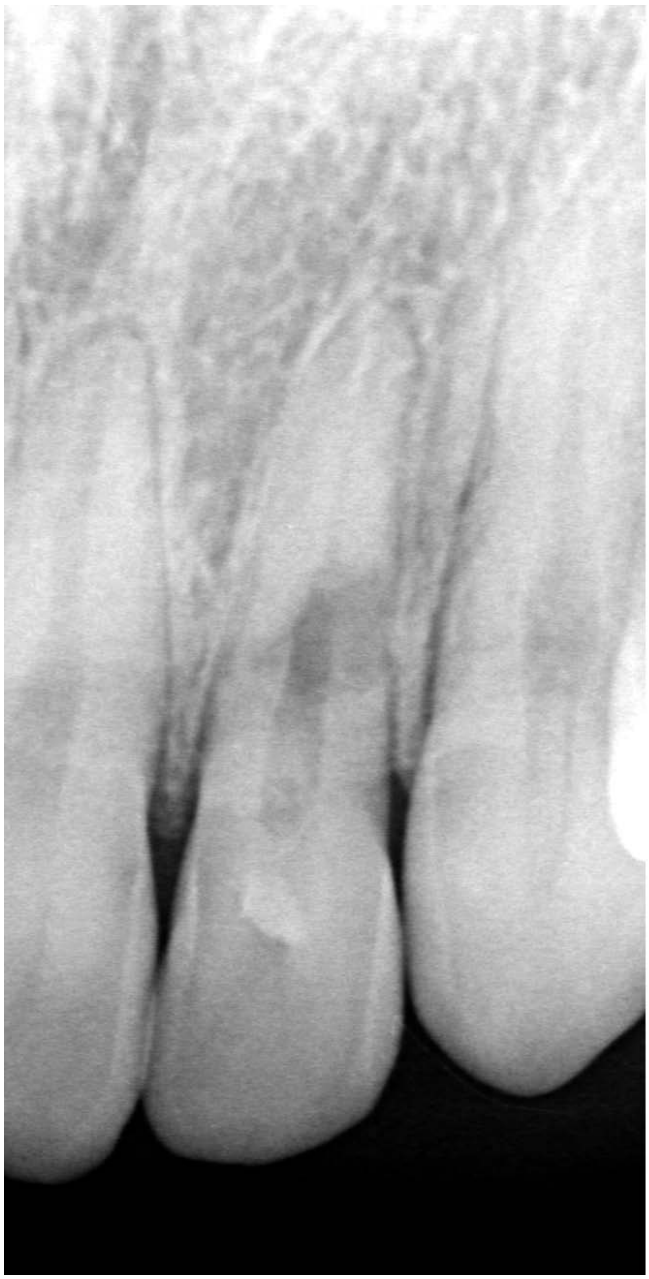
In favor of external resorption was the shape of the defect, with the wider defect facing the bone, the shape of the defect was not rounded, which would be characteristic of internal resorption.

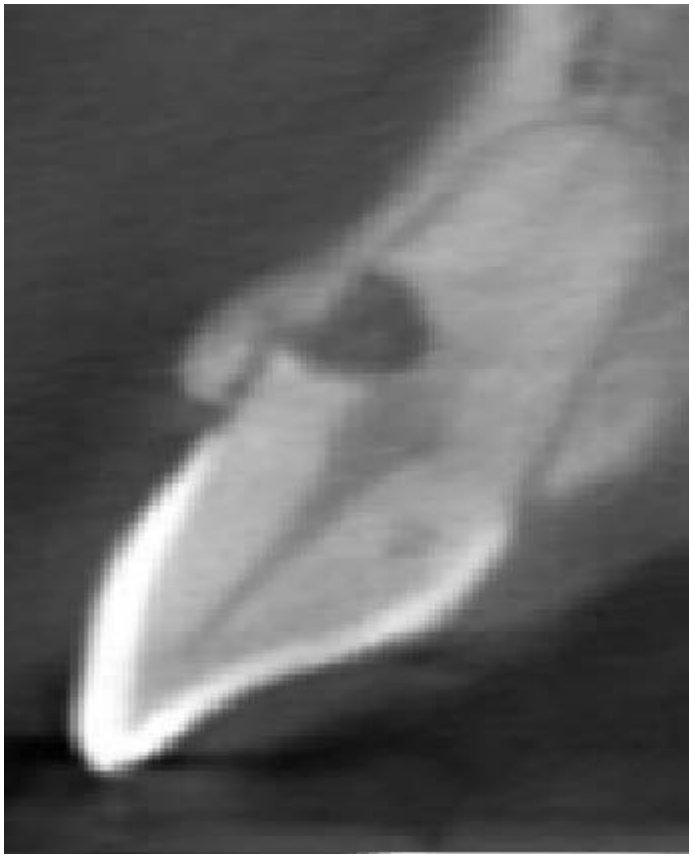
Also, there were signs in favor of internal resorption. The defect was below the cervical part, which is not typical of external cervical resorption. The response to cold stimuli reduced, which is not characteristic of external resorption, as it affects the pulp only in the last stages of tooth structures decay. Furthermore, the X-ray obliteration of the root canal beyond the resorption area is not characteristic of external resorption.

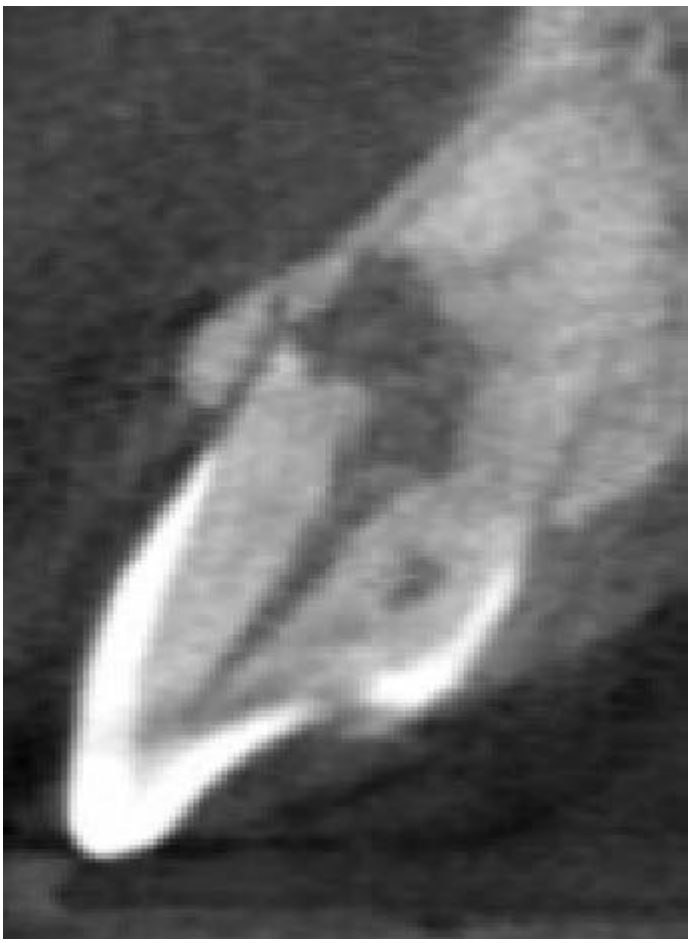
*Visit 1:* Pre-op X-ray plus anesthesia with sol. Ubisthesini 4% - 1 ml, isolation with rubberdam. The access was made as close as possible to the incisal edge. When opened, at first glance the pulp chamber looked quite

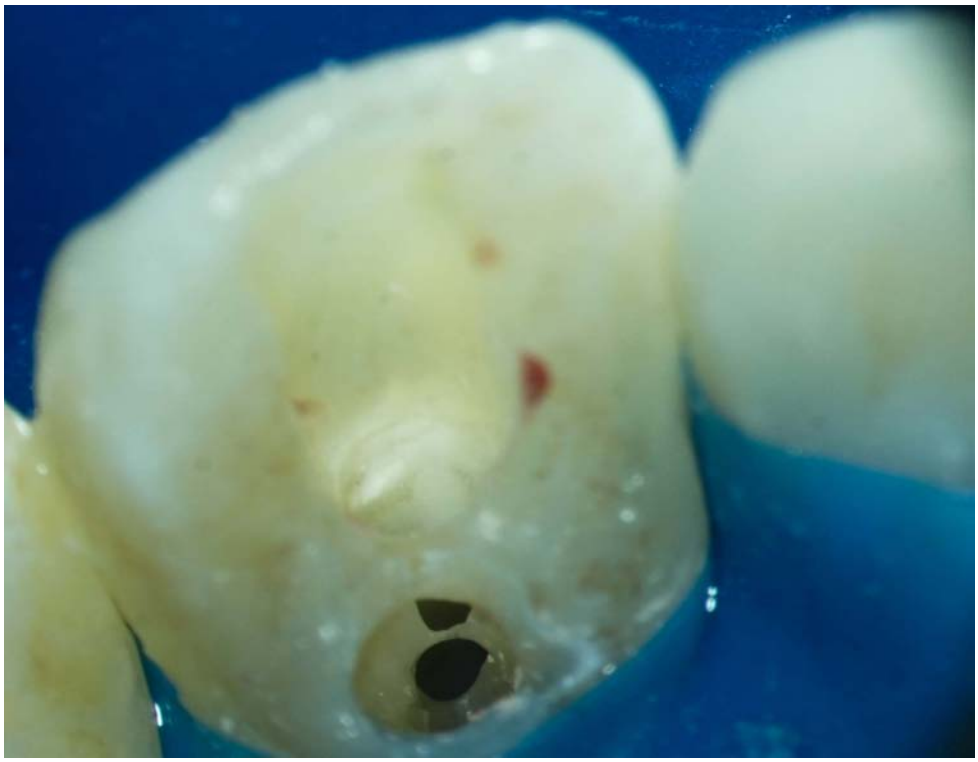
vital. But after passing the canal orifice, a heavy bleeding started and granulation tissue was found. Another access was made through the invagination. Both the invagination canal and the main one converged at the orifice. The visit was completed by irrigation with NaOCl 5.25%, and obturation with Ca(OH)2 to reduce granulation tissue volume.

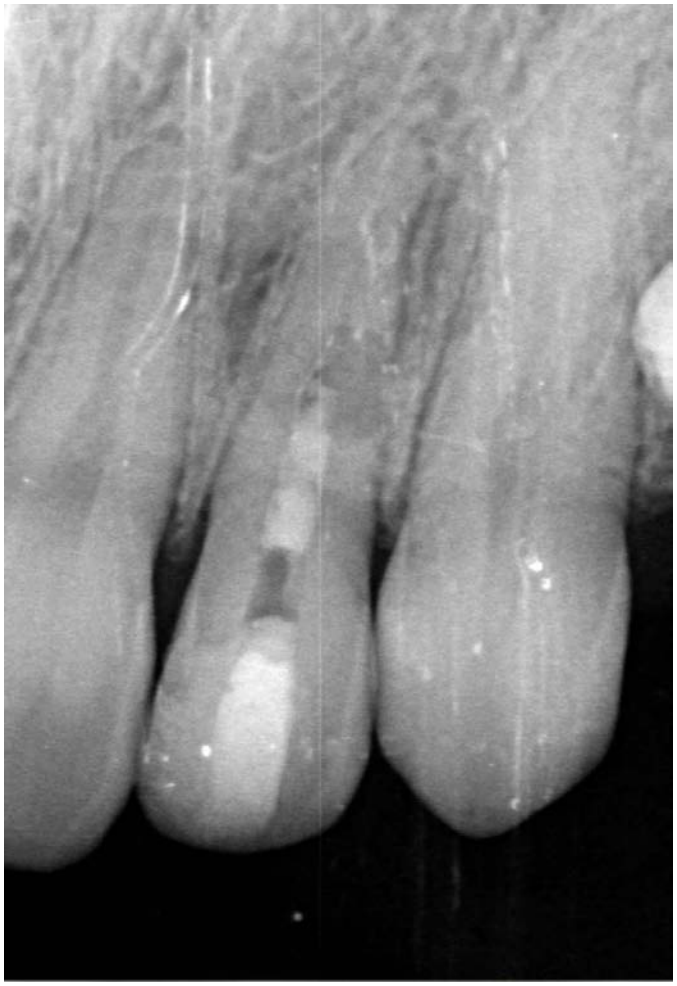
Pics 1,2,3,4,5





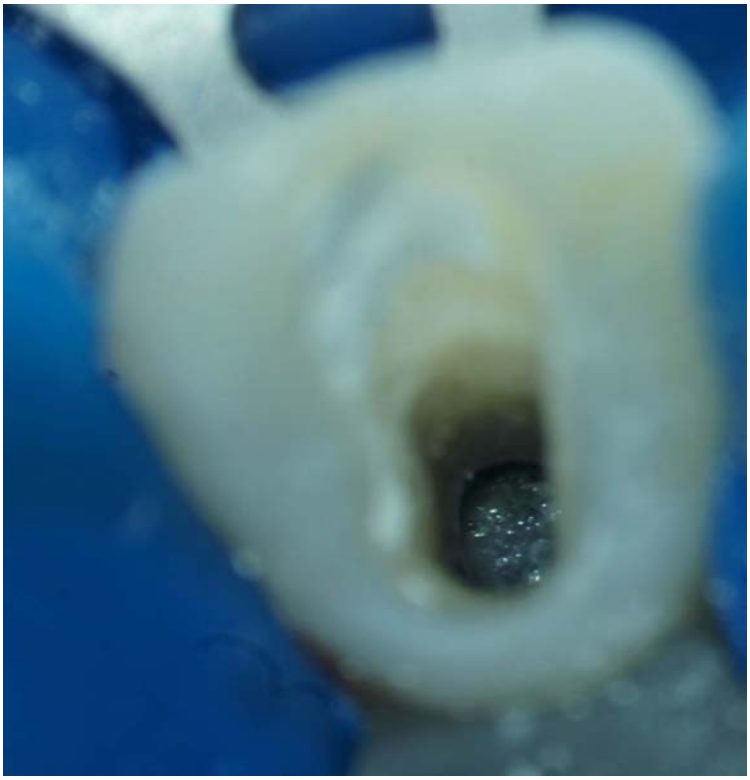


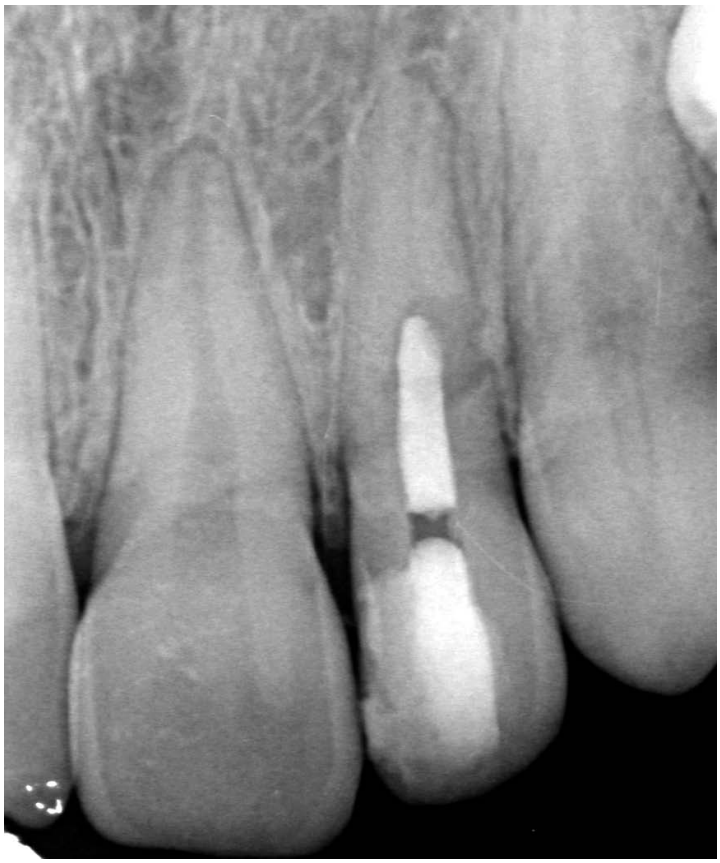




*Visit 2:* In 2-week time, treatment was carried out with NaOCl 5.25%, followed by obturation with Ca (OH)2 due to inability to dry the root canals; though no bleeding was observed at the second visit, exudation persisted.

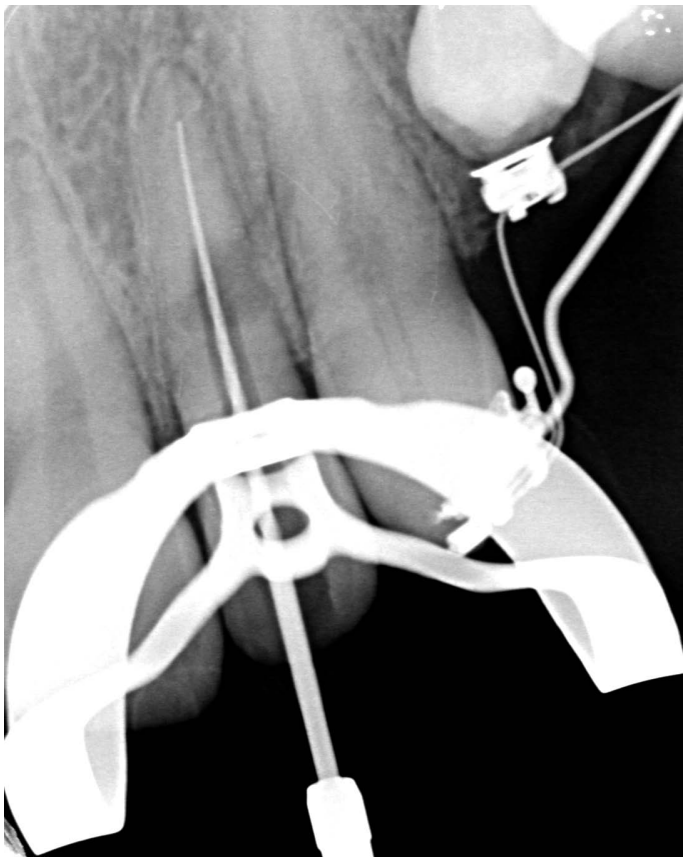
Pics 6,7

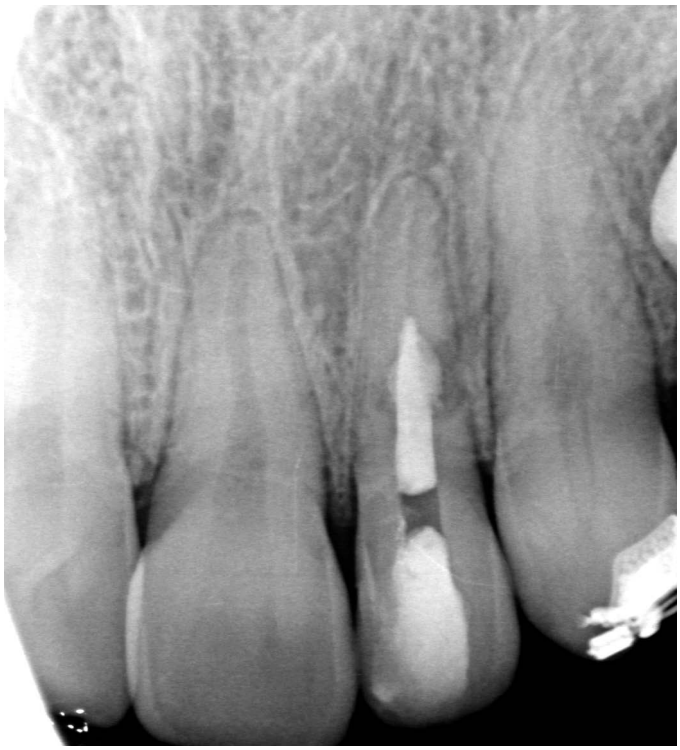




*Visit 3:* Took place4 weeks after the start of treatment. During the visit the apical part of the root canal was accessed. The entrance to the apical part was closed, i.e. obliterated. The access had to be carried out using a DTE ultrasound scaler and a SANI U file size 20. The final instrumental processing of the apical part was performed with Soco Sc 35-04 files. Due to the complex anatomy and the lack of time, the final obturation was postponed to the next visit.

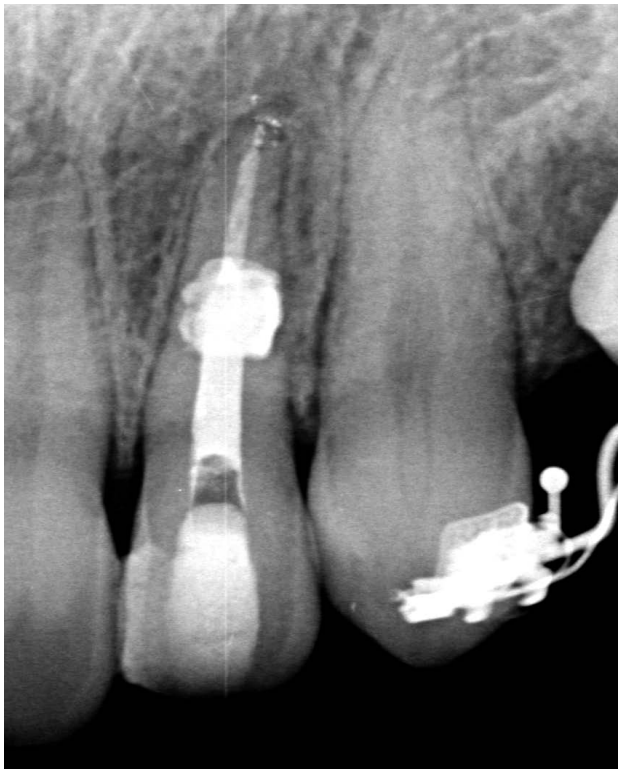
Pics 8,9





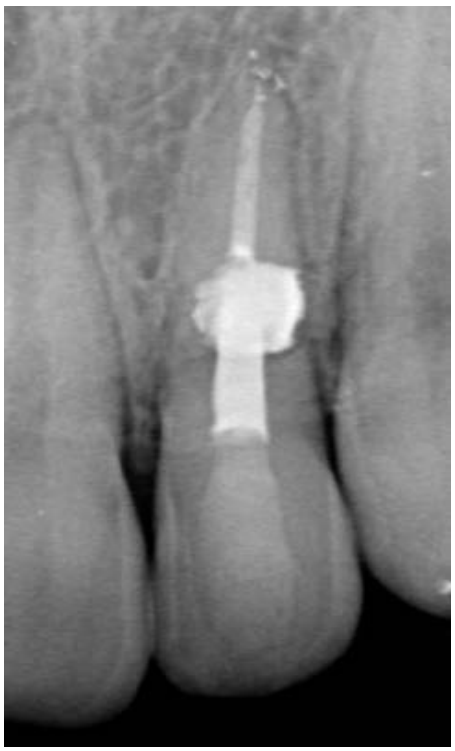
*Visit 4:* Took place in 6 weeks since the treatment beginning. The apical third was obturated using vertical condensation of gutta-percha. The root was sealed with liquid.
composite, followed by treatment with NaOCl 5,25 % and obturation with Ca(OH)2.

Pic 10



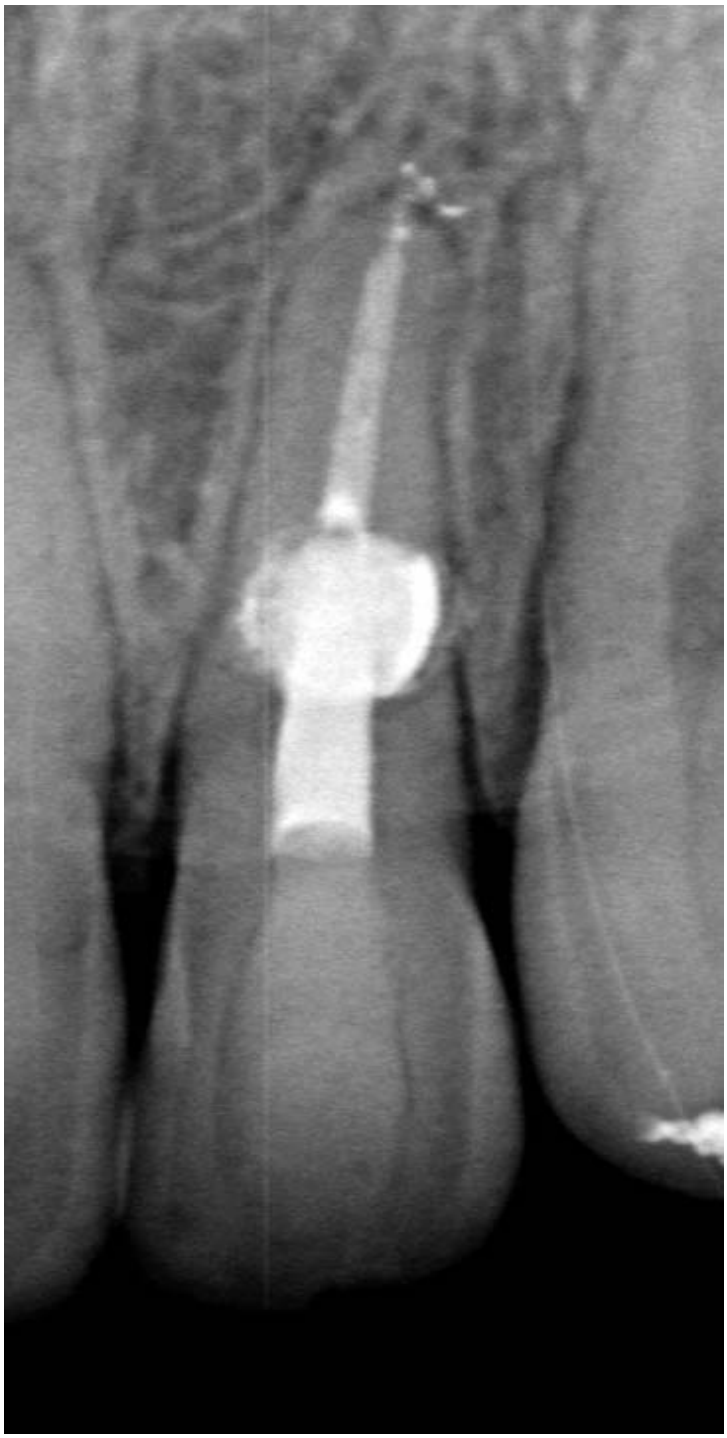
*Visit 5:* The root canal was obturated with biocearamic sealer and a central pin. The approach was chosen only owing to the fluid properties of the sealer. Inserting and adapting MTA would have been extremely challenging under those conditions.

Pic 11



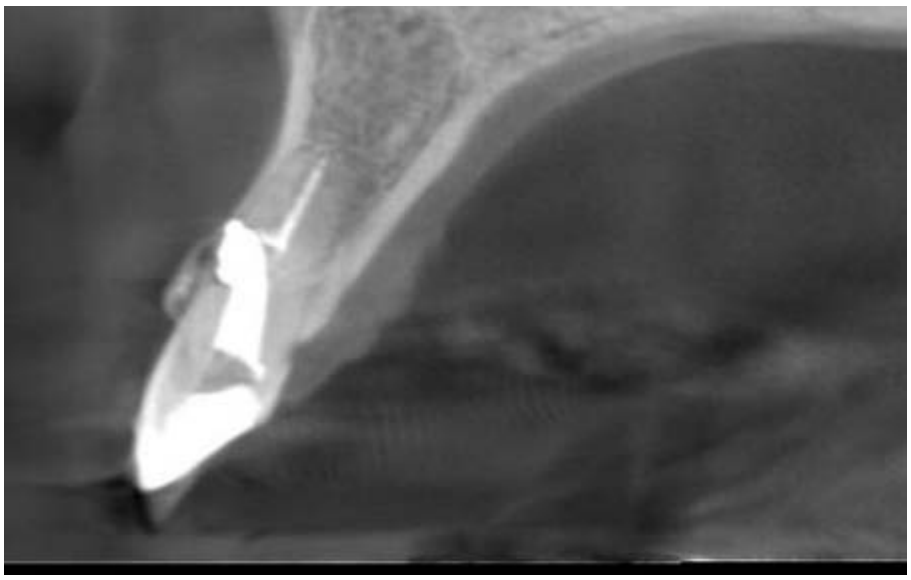
*Visit 6:* Ten weeks passed since the beginning of the treatment. Material hardening was controlled, and a permanent filling was inserted.

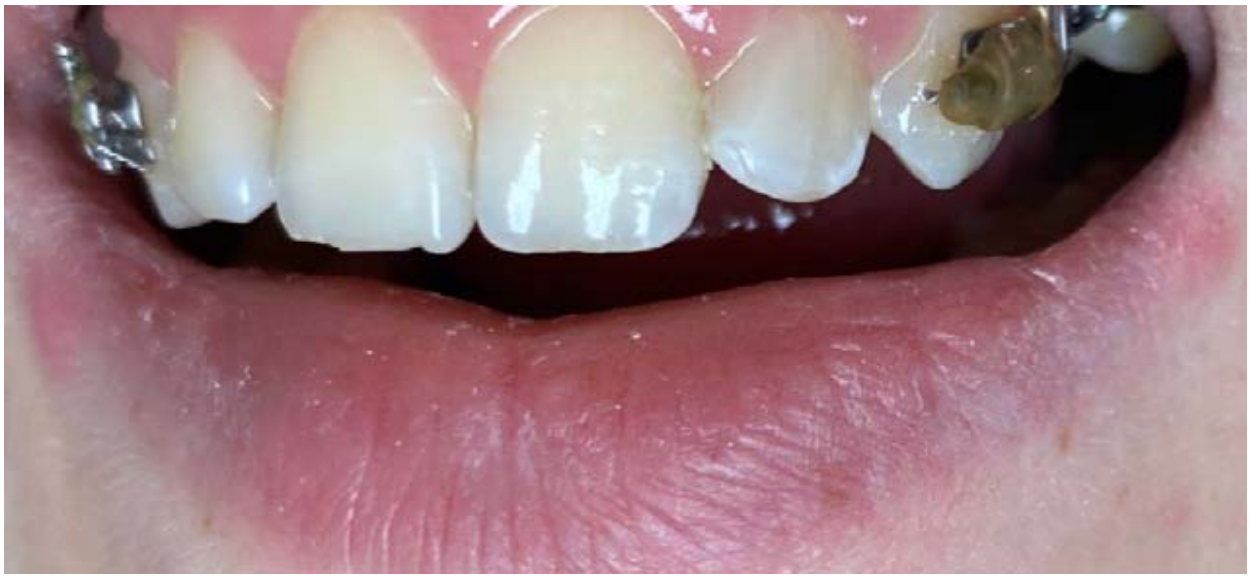
Pic 11a



Post-op recall in 1 year

Pic 12,13.





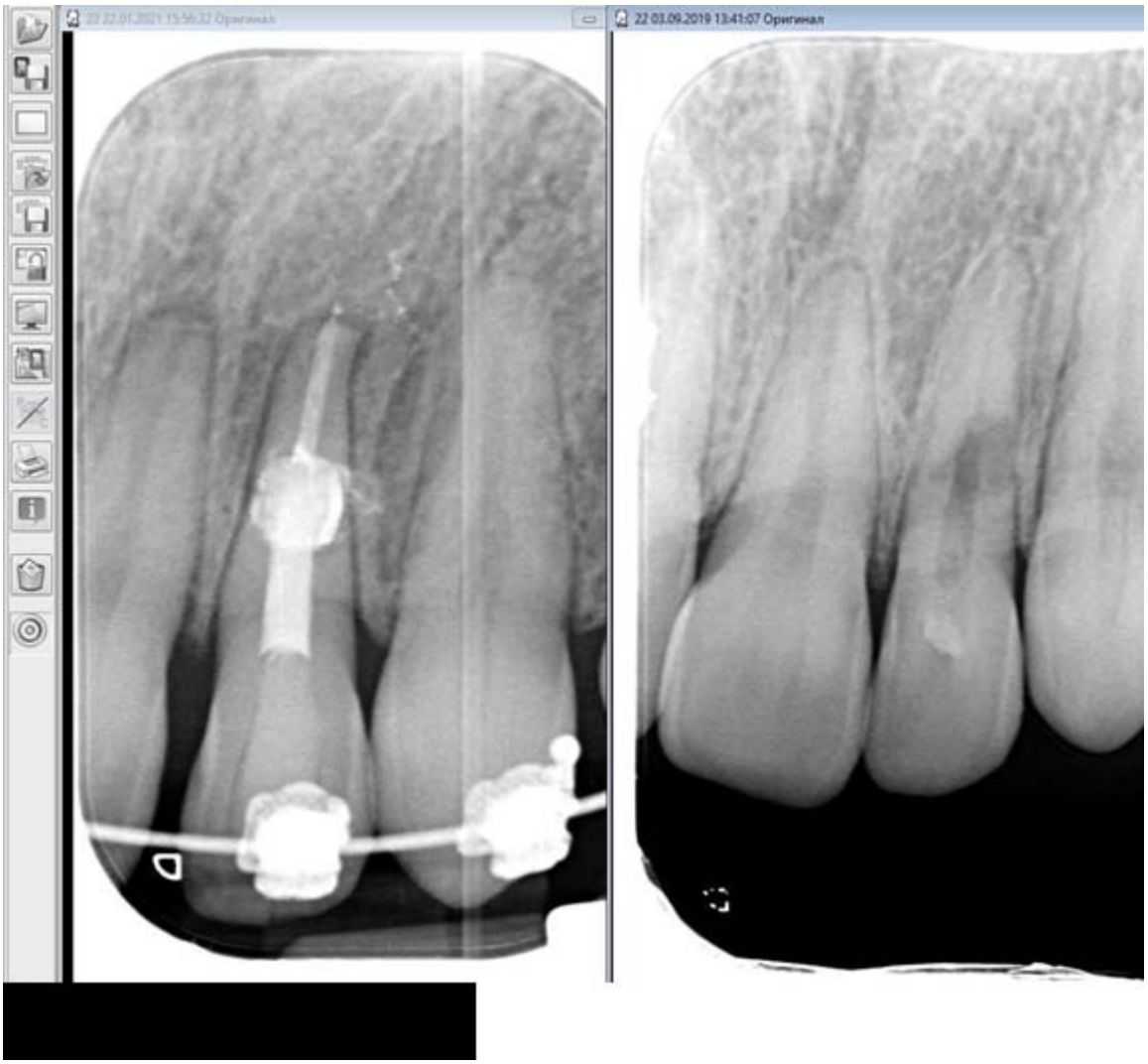
## II. Response to the Endodontic Treatment

The patient started orthodontic treatment, however, tooth 2.2 was temporarily not included in the orthodontic therapy at the endodontist's request, who was willing to observe it for a year. Furthermore, increased resorption could have been provoked. As of today, the tooth is included in the orthodontic treatment and is being followed up.

In eighteen-month time, the stabilized process is observed, meaning that the diagnosis has been correct and the manual work has been performed without problems.

No complaints are observed.

Pic 14




## III. Conclusions

The difference between internal and external resorption lies in the fact that high-quality removal of granulation tissue by mechanical and chemical (calcium hydroxide) techniques allows for achieving a high level of recuperation. Also, an accurate DI diagnosis makes it possible to seal the invaginated area at the early stages before pulp-associated complications occur, which would later require comprehensive endodontic treatment. Other approaches and tactics for treating teeth with invaginations are described in previous articles by the authors.

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# Recent Host Modulation Therapy: A Mini Review

By Sakshi Gaind & Tushar Pruthi

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*Introduction-* Periodontitis is one of the most ubiquitous diseases and is characterized by the destruction of connective tissue and dental bone support following an inflammatory host response secondary to infection by periodontal bacteria.[1] The first clinical manifestation of periodontal disease is the appearance of periodontal pockets, which offer a favorable niche for bacterial colonization. It can be diagnosed by clinical examination with periodontal probe to determine pocket depths in combination with X-ray imaging, using microbiological techniques for a precise analysis of the infectious agents.[2]

According to current concepts of the multifactorial etiology of periodontal disease, it is caused by the interaction among single or multiple microbial agents, a host with some degree of susceptibility, and environmental factors with an influence on both. Although a single model of the etiopathogeny of periodontal disease has yet to be validated, it is broadly accepted that periodontal disease results from action of the bacterial biofilm on the periodontium of the susceptible individual. Bacteria are able to survive and grow in the complex ecosystem of this biofilm because of their production of virulence factors. These factors also confer a greater resistance to host defense mechanisms, i.e., they increase the capacity of the bacteria to overcome the inflammatory reaction and immune response to antigen presentation.[3]

*GJMR-J Classification:* *NLMC Code: WU 29*


*Strictly as per the compliance and regulations of:*

# Recent Host Modulation Therapy: A Mini Review

Sakshi Gaind [α] & Tushar Pruthi [σ]

## I. Introduction

Periodontitis is one of the most ubiquitous diseases and is characterized by the destruction of connective tissue and dental bone support following an inflammatory host response secondary to infection by periodontal bacteria.[1] The first clinical manifestation of periodontal disease is the appearance of periodontal pockets, which offer a favorable niche for bacterial colonization. It can be diagnosed by clinical examination with periodontal probe to determine pocket depths in combination with X-ray imaging, using microbiological techniques for a precise analysis of the infectious agents.[2]

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According to a review by Offenbacher in 1996, the presence of bacteria in the periodontal pocket triggers a reaction that starts with intervention of the neutrophilantibody-complement axis, stimulating different cell types.[4]

Host modulatory therapy is a new treatment modality that has been incorporated into the dental therapeutics but it has not been well implemented in the dental practice due to the easy unavailability of host modulatory agents in India. Host can be defined as "the organism from which a parasite obtains nourishment," or in the transplantation of tissue, "the individual who receives the graft". Modulation is defined as "the alteration of function or status of something in response to a stimulus or an altered chemical or physical environment". Host modulation with chemotherapeutic agents or drugs is a promising new adjunctive therapeutic opportunity for the management of periodontal diseases. The concept of host modulatory therapy was first introduced to dentistry by Williams and Golub et. al. and then expanded by many other researchers in the dental profession. Golub and colleagues discussed "host modulation with tetracyclines and their chemically modified analogues".[5]

Three potential approaches to host modulation have been considered: 1) inhibition of matrix metalloproteinases (MMPs) with antiproteinases, 2) blocking production of proinflammatory cytokines and prostaglandins with antiinflammatory drugs, and 3) inhibiting activation of osteoclasts with bone-sparing agents.[6]

## II. Host Response

Concepts of the etiology of periodontal disease have changed noticeably in the last four decades. In 1985 research began to focus on bacterial-host interactions. Several specific subgingival oral bacteria including porphyromonus gingivalis, actinobacillus aggregatibater, prevotela intermedia, bacteroides forsythus and perhaps others such as campylobacter rectus, fusobacterium nucleatum, and spirochetes are associated with severe type of periodontal diseases.[7] Protective aspects of the host response include recruitment of neutrophils, production of protective antibodies, and possibly the release of antiinflammatory cytokines including transforming growth factor (TGF-β), interleukin-4 (IL-4), IL-10, and IL-12. Persistent bacterial aggression disrupts homeostatic mechanisms and results in release of proinflammatory cytokines (e.g., IL-1, IL-6, tumor necrosis factor- α (TNF-α), proteases (e.g., Matrix Metallo proteinase's), and prostanoids (e.g., prostaglandin E2 [PGE2]), which can endorse extracellular matrix destruction in the periodontium and stimulate bone resorption, tooth mobility and tooth loss.[8]

## III. Host Modulation

The therapeutical agents or perioceutics that are mainly used to control periodontitis is a rising branch in the treatment of periodontal diseases along with mechanical debridement. To lower excessive levels of enzymes, cytokines, prostanoids (prostaglandin E2 [PGE2]), as well to modulate osteoclast functions, host modulation therapy (HMT) are being used, but it should not reduce below constitutive levels. Nonsteroidal anti-inflammatory drugs (NSAIDS), subantimicrobial dose doxycycline (Periostat), systemic bisphosphonates (BP), are few host modulating agents that are being

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recommended. Systemic flurbiprofen and topical ketoprofen are NSAIDS that act by inhibiting PGE2. Bisphosphonates modulates the osteoclast function, and subantimicrobial dose doxycycline uses the anticollagenase properties of tetracycline (TC), which is lone permitted drug by FDA. Future prospect lies for chemically modified tetracycline (CMT's), bone resorption uncouplers, anti cytokine drugs, antimetabolites, and lipoxins (LXs). This provides clinician with supplementary equipment to conventional mechanical debridement, which could improve and make the clinical therapeutic outcome more predictable, in a susceptible host.[9] In addition, a number of local host modulatory agents have been investigated in clinical trials for their potential use as adjuncts to surgical procedures not only to improve upon wound healing but also to stimulate regeneration of lost bone, periodontal ligament, and cementum, restoring the complete periodontal attachment apparatus. These have included enamel matrix proteins (Emdogain), bone morphogenetic proteins (BMP-2 and BMP-7), growth factors (platelet-derived growth factor and insulin-like growth factor), and tetracyclines. The only host modulatory agent currently approved by the FDA for adjunctive use during surgery is Emdogain.[10]

## IV. Classification of the Various Host Modulation Therapies

A. Kenneth S. Kornman, 1999[11]

i. Host Modulation

1. Blocking Direct Effectors of Bone and Connective Tissue Destruction E.g. bisphosphonates, MMP inhibitors ii. Host Modulation
2. Blocking Host Mechanisms That Influence Clinical Outcomes E.g. NSAIDs, inhibitors of IL-1 and TNF iii. Host Modulation
3. Host Mechanisms That Influence Bacterial Control E.g. agents that reduce levels of PGE2 , IL-1,TNF

B. Reddy MS, Geurs NC, Gunsolley JC, 2003[12]

i. Anti-proteinases - E.g.: tetracyclines
ii. Anti-inflammatory agents - E.g. NSAIDs
iii. Bone sparing agents - E.g. Bisphosphonates

C. Anarthe RD, Mani DA, Marawar DPP, 2013[13]

a) Inhibition of matrix metalloproteinase (MMPs): This is achieved by chemically modified tetracyclines (CMTs)

b) Inhibition of arachidonic acid metabolite: Through NSAIDs

a. COX-1 inhibitors: Indomethacin, Flurbiprofen, Naproxen.
b. COX-2 inhibitors: Rofecoxib.
c. COX and LOX inhibitors: Triclosan, Topical ketoprofen.
d. LOX inhibitors: Lipoxins.

c) Modulation of bone metabolism

a. Bisphosphonates
b. Hormone replacement therapy (HRT)
c. Calcium supplementation.

d) Regulation of immune and inflammatory response:

a. Suppressing pro-inflammatory cytokines: IL1 and TNF-α receptor antagonist.
b. Nitric oxide inhibition.
c. Generation of protective antibodies through vaccination.
d. Infusion/ supplementary anti-inflammatory cytokines: IL-4 and IL-10.

D. Carranza, Newman, Takei, Klokkevold[14]

i. Systemically administered agents- NSAIDs, Bisphosphonates, Subantimicobial-dose doxycycline (SDD)
ii. Locally administered agents; NSAIDs, Enamel matrix proteins (EMP), Growth factors, Bone morphogenetic proteins.

## V. Chemically Modified Tetracyclines

Tetracyclines were first introduced in 1948 and were soon recognized as highly effective against Rickettsiae, several Gram-positive and Gram-negative bacteria, and other organisms. These nonantibiotic tetracyclines analogs are nothing but the tetracycline molecules which have been modified to eliminate the antimicrobial property, but retain the host modulatory, anticollagenolytic property. Furthermore, these drugs, known as broad spectrum antibiotics.[15]

Chemically Modified Tetracyclines are used as Host Modulating agents in the management of periodontitis by inhibition of Matrix Metalloproteinases, inhibition of proinflammatory cytokines, inducible nitric oxide synthase (iNOS) and inhibition of bone resorption, enhancement of the attachment of fibroblasts and connective tissues to the tooth surface.[16] The anti- Matrix Metalloproteinases actions of Chemically Modified Tetracyclines include direct inhibition of the active MMPs by the virtue of Ca2+ and Zn2+-binding sites, inhibition of reactive oxygen species-mediated activation of pro-MMPs, proteolysis of pro-MMPs into enzymatically inactive fragments, protection of α-1 proteinase inhibitor from MMPs, reduction in the activity of serine proteinases. Polymorphonuclear leucocytes (PMNs) provide the major source of collagenases that mediate the connective tissue breakdown during inflammatory periodontal disease, while the fibroblasts contribute the collagenase required for connective tissue remodeling in normal gingiva. The anti-collagenase activity of CMTs is specific against the collagenase produced from neutrophils but not the fibroblasts.[16]

*a) SDD-Sub antimicrobial dose of Doxycycline*

SDD is the only systemic host response modulator specifically indicated as adjunctive treatment for periodontitis and it is approved by USFDA and UK medicines and health care products regulatory agency. It is marketed as periostat, 20mg dose of doxycycline hyclate BD for 3-9 months has the ability to down regulate MMPs.[17]

Mechanism of Action.[11]

1. In junctional epithelium inhibition of production of epithelial derived MMPs by inhibiting cellular expression and synthesis.
2. In connective tissue - Direct inhibition of active MMPs by cation chelation Inhibition of oxidative activation of latent MMPs, down regulates the expression of key inflammatory cytokines including interleukin IL1,IL6, and tumor necrosis factor (TNF)α, as well as prostaglandin E2 (PGE2)Scavenges and inhibits production of reactive oxygen species (ROS) produced by PMNs (e.g. HOCl, which activates latent MMPs),Inhibition of MMPs and ROS protects α1 proteinase inhibitor (α1PI) thereby indirectly reducing tissue proteinase activity, Stimulates fibroblast collagen production.
3. Alveolar bone- Reduces osteoclast activity and bone resorption, blocks osteoclast MMPs, Stimulates osteoblast activity and bone formation.

Crout et al. 1996 - In a study of 14 patients with chronic periodontitis, after removal of subgingival plaque and calculus, patients were randomised to receive either SDD for 2 months, then no drug for 2 months, then SDD for 2 months or placebo for 2 months, then no drug for 2 months, then placebo for 2 months SDD resulted in significantly improved probing depths and attachment levels compared with placebo, but did not affect plaque index or gingival inflammation.[18]

Al-Shammari et al. 2001 - SDD was given to 12 patients with chronic periodontitis for 2 months following a course of subgingival instrumentation. Six patients were prescribed placebo. At baseline, months 1 and 2, GCF samples were collected and analysed for MMP-8, MMP-13 and ICTP (carboxyterminal peptide, a pyridinoline-containing fragment of type-1 collagen). The 2-month regime of SDD resulted in statistically significant reductions in GCF concentrations of ICTP, MMP-8 and MMP-13 compared with placebo. This was the first study that demonstrated in human subjects that SDD results in a simultaneous reduction of elevated MMP activity with a concomitant reduction in levels of collagen degradation fragments. SRP alone has no effect on GCF ICTP levels.[18]

## VI. Bisphosphonates

The bisphosphonates are bone-seeking agents that inhibit bone resorption by disrupting osteoclast activity.[11]

Mechanism of action

Bisphosphonates acts on osteoclast function at Tissue, Cellular and molecular levels

1. Tissue level: Decrease bone turnover due to decreased bone resorption, Decreased number of bone multicellular units, Net positive whole body bone balance
2. Cellular level: Decreased osteoclast recruitment, Increased osteoclast apoptosis, Decreased osteoclast adhesion, Increased osteoblast differentiation and number
3. Molecular level: Inhibit mevalonate pathway, Decreased post translational phenylation of GTPbinding proteins.[19]

Rocha et al. used oral route of alendronate as host modulating agent and found that there is decreased alveolar bone resorption, decreased tooth mobility and decreased clinical parameters.[20]

Pradeep AR et al. used Alendronate as local drug delivery as 1% gel and found that there is increase percentage of bone fill, decreased probing depth and clinical attachment level.[21]

*Other host modulatory agents*

i. Probiotics

Probiotics have demonstrated significant potential as therapeutic options for a variety of disease as they have been known to modulate cytokine secretion profiles, influence TR09;lymphocyte populations, protect against physiologic stress, and enhance intestinal epithelial cell function and antibody secretion.[22] Teughels et al. explored the use of probiotics in influencing the periodontal microbiota and periodontal health and concluded that probiotics might offer opportunities to manipulate the oral microbiota, and periodontal health by either direct microbiological interactions or by immunomodulatory interactions.[23]

ii. Periodontal Vaccine

George Hajishengallis reported that toll like receptors (TLRs) may offer novel targets for hostR09; modulation therapy in periodontitis since manipulation of TLR signalling may contribute to control of infection or regulation of inflammation and, moreover, synthetic or natural TLR agonists could serve as novel periodontal vaccine adjuvants.[24]

## VII. Summary & Conclusion

The improved understanding of the host bacterial interactions and the host immune inflammatory response leading to periodontal tissue destruction has led to the development of Host Modulation Therapy.

Subantimicrobial dose doxycycline (SDD) is the only HMT currently approved and indicated as an adjunct to SRP for treating periodontitis. In the future a range of HMTs targeting different aspects of the destructive cascade of breakdown events in the periodontal tissues are likely to be developed as adjunctive treatments for periodontitis. The further development of these agents will permit dentists to treat specific aspects of the underlying biochemical basis for periodontal disease. The goal is to maximize the treatment response by reducing inflammation and inhibiting destructive processes in the tissues, which will result in enhanced periodontal stability after conventional periodontal treatments such as SRP.

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# Global Journals Guidelines Handbook 2021

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# Memberships

## Fellows/Associates of Medical Research Council

### FMRC/AMRC Memberships

## Introduction

FMRC/AMRC is the most prestigious membership of Global Journals accredited by Open Association of Research Society, U.S.A (OARS). The credentials of Fellow and Associate designations signify that the researcher has gained the knowledge of the fundamental and high-level concepts, and is a subject matter expert, proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice. The credentials are designated only to the researchers, scientists, and professionals that have been selected by a rigorous process by our Editorial Board and Management Board.

Associates of FMRC/AMRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

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### Fellow of Medical Research Council

FELLOW OF MEDICAL RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.

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Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

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A FMRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation


## Certificate

### Certificate, LoR and Laser-Momento

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Fellows can use the honored title of membership. The “FMRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FMRC or William Walldroff, M.S., FMRC.

Career | Credibility | Exclusive | Reputation

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Career | Financial

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Associates can publish articles (limited) without any fees. Also, they can earn up to 30-40% of sales proceeds from the sale of reference/review books/literature/publishing of research paper

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Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

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| Associate | Fellow | Research Group | Basic |
|---|---|---|---|
| $4800<br>**lifetime designation** | $6800<br>**lifetime designation** | $12500.00<br>**organizational** | APC<br>**per article** |
| **Certificate,** LoR and Momento<br>**2** discounted publishing/year<br>**Gradation** of Research<br>**10** research contacts/day<br>**1 GB** Cloud Storage<br>**GJ** Community Access | **Certificate,** LoR and Momento<br>**Unlimited** discounted publishing/year<br>**Gradation** of Research<br>**Unlimited** research contacts/day<br>**5 GB** Cloud Storage<br>**Online Presense** Assistance<br>**GJ** Community Access | **Certificates,** LoRs and Momentos<br>**Unlimited** free publishing/year<br>**Gradation** of Research<br>**Unlimited** research contacts/day<br>**Unlimited** Cloud Storage<br>**Online Presense** Assistance<br>**GJ** Community Access | **GJ** Community Access |

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**We accept the manuscript submissions in any standard (generic) format.**

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

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Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

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3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
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- Writings
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- Any other original work

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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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**Acknowledgments**

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

***Manuscript Style Instruction (Optional)***

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

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***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

**Author details**

The full postal address of any related author(s) must be specified.

**Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

**Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

**Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

**Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

**Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

**Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

**Figures**

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

## Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

## Tips for writing a good quality Medical Research Paper

***1. Choosing the topic:*** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

***2. Think like evaluators:*** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

***3. Ask your guides:*** If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

***14. Arrangement of information:*** Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

***15. Never start at the last minute:*** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

***16. Multitasking in research is not good:*** Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

***17. Never copy others' work:*** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

***18. Go to seminars:*** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

***19. Refresh your mind after intervals:*** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

***20. Think technically:*** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

***21. Adding unnecessary information:*** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

***22. Report concluded results:*** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

***23. Upon conclusion:*** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

## Informal Guidelines of Research Paper Writing

***Key points to remember:***

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.

*Mistakes to avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

**Title page:**

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

*Reason for writing the article—theory, overall issue, purpose.*

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

**Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

**Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

## The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection*.

*Segment draft and final research paper:* You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

*Written material:* You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

## Criterion for Grading a Research Paper (Compilation) by Global Journals

**Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.**

| Topics | Grades | | |
|---|---|---|---|
| | **A-B** | **C-D** | **E-F** |
| ***Abstract*** | Clear and concise with appropriate content, Correct format. 200 words or below | Unclear summary and no specific data, Incorrect form Above 200 words | No specific data with ambiguous information<br>Above 250 words |
| ***Introduction*** | Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited | Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter | Out of place depth and content, hazy format |
| ***Methods and Procedures*** | Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads | Difficult to comprehend with embarrassed text, too much explanation but completed | Incorrect and unorganized structure with hazy meaning |
| ***Result*** | Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake | Complete and embarrassed text, difficult to comprehend | Irregular format with wrong facts and figures |
| ***Discussion*** | Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited | Wordy, unclear conclusion, spurious | Conclusion is not cited, unorganized, difficult to comprehend |
| ***References*** | Complete and correct format, well organized | Beside the point, Incomplete | Wrong format and structuring |

# INDEX

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