Global Journal of Medical Research: J
Dentistry & Otolaryngology
Volume 21 Issue 2 Version 1.0 Year 2021
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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

Hassan II University

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*.

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%. 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

Corresponding Author α: Orthodontist, private practice, PhD student, Physiopathology molecular genetics and biotechnologies laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, MOROCCO. e-mail: fatenenassiba@gmail.com

Author σ: PhD, Physiopathology molecular genetics and biotechnologies laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, MOROCCO.

Author ρ ¥: Professor, Physiopathology molecular genetics and biotechnologies laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, MOROCCO.

Author ω: Professor, director of Physiopathology Molecular Genetics and Biotechnologies laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, MOROCCO.

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.

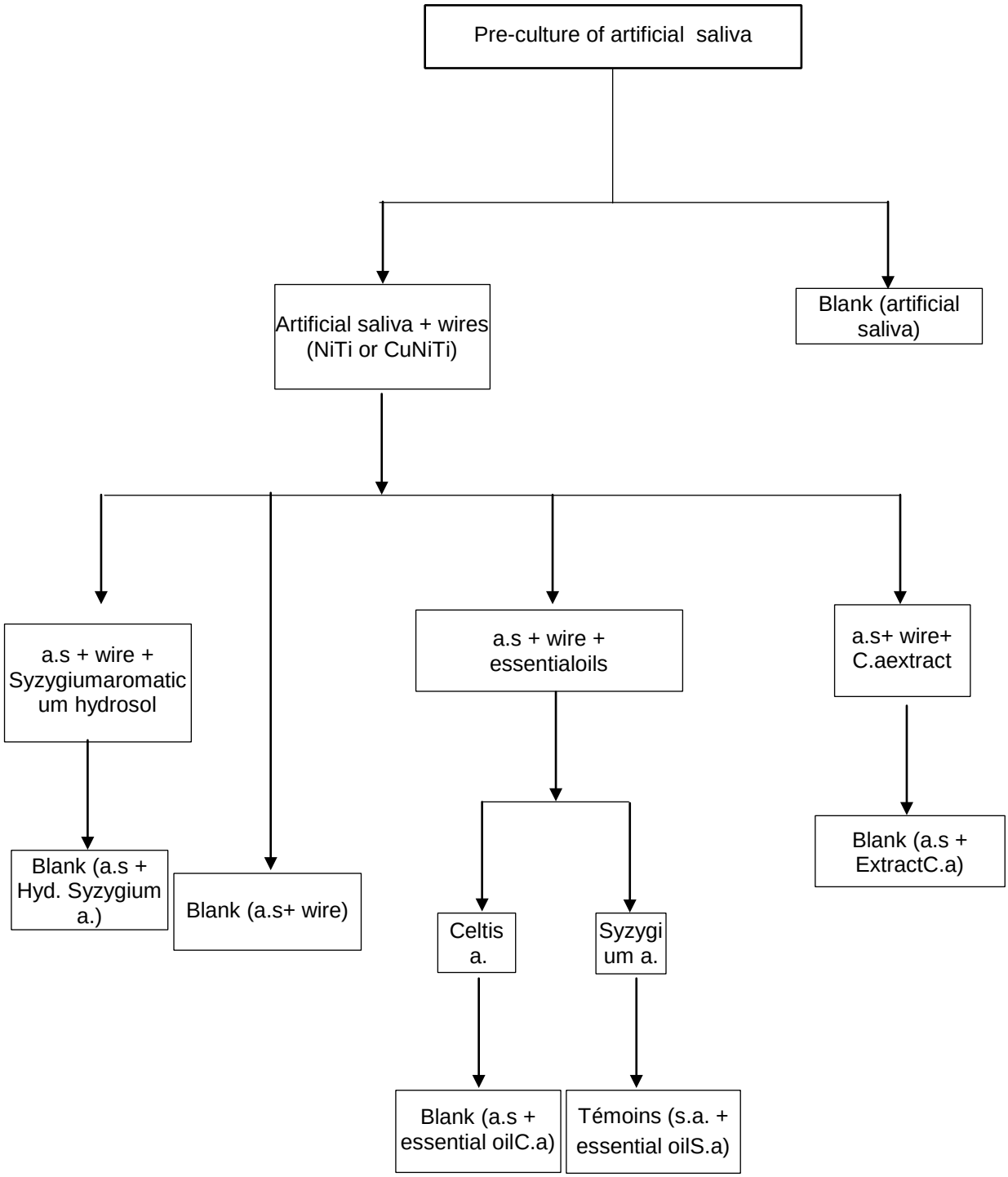

Figure 1: Distribution of different solutions and negative and positive controls. s.a: artificial saliva, NiTi: Nickel-Titanium, CuNiTi: Copper-Nickel-Titanium, S.a: Syzygium aromaticum, C.a: Celtis australis, Hyd: hydrosol.

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).

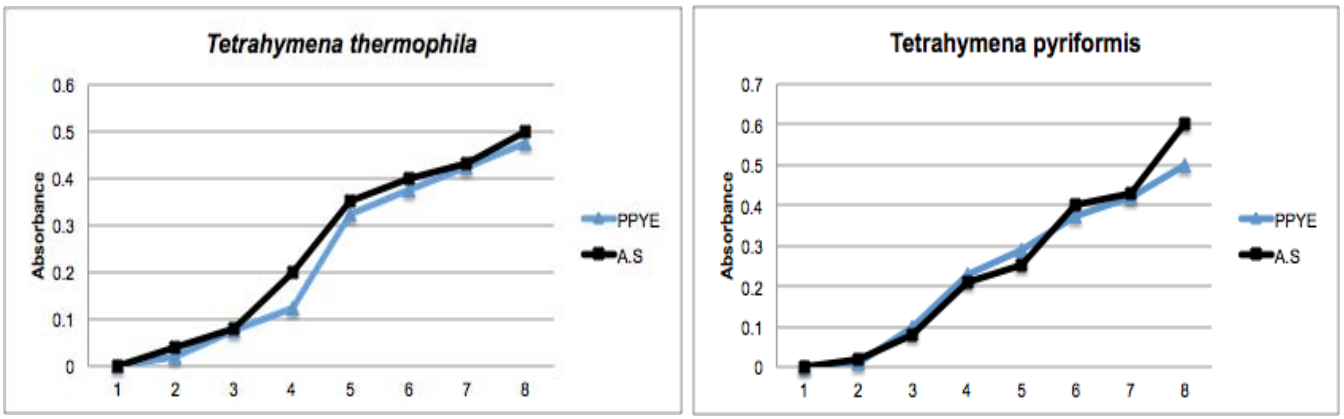

Figure 2: Growth kinetics of Tetrahymena thermophila and Tetrahymena pyriformis in artificial saliva and PPYE medium during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva

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).

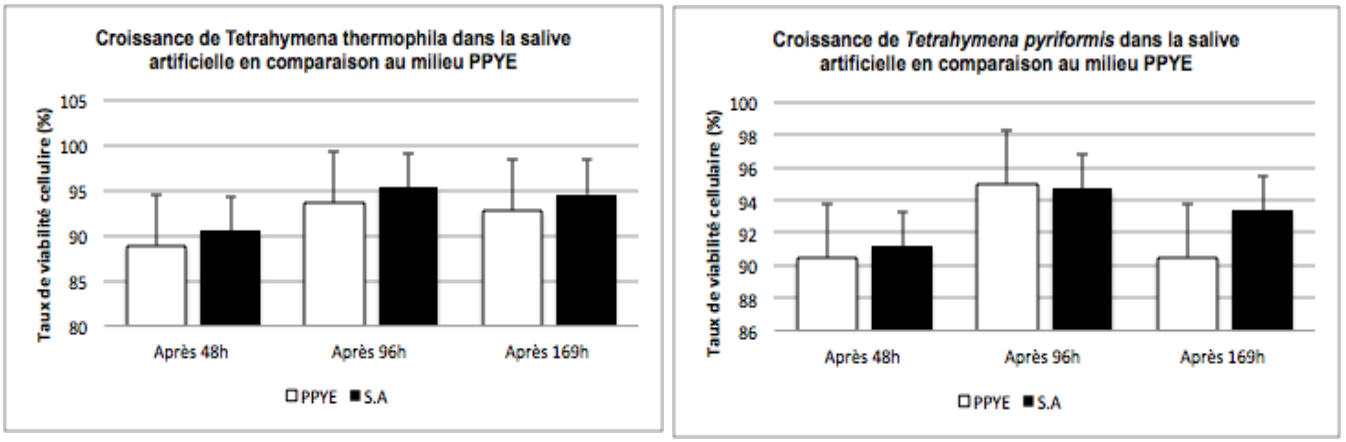

Figure 3: Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in artificial saliva (S.A) and the PPYE medium. There is no statistically significant difference between the two environments.

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).

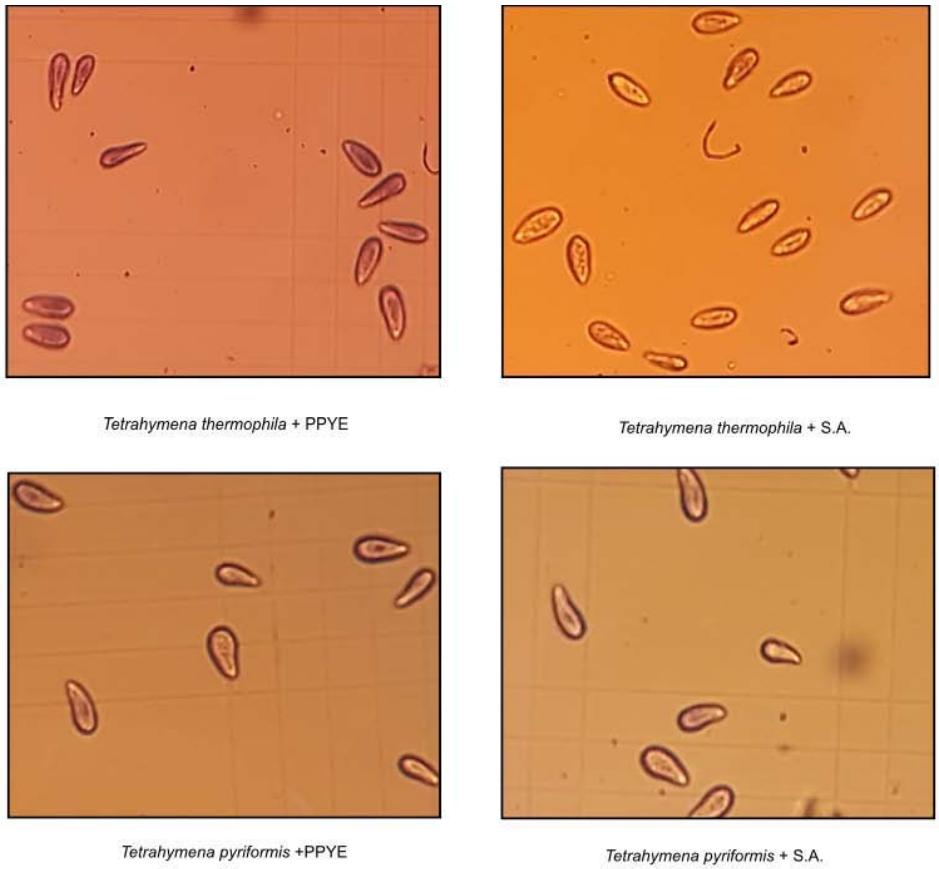

Figure 4: Microscopic images of Tetrahmyena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 100 magnification, showing the comparison of growth in artificial saliva and the usual medium PPYE

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).

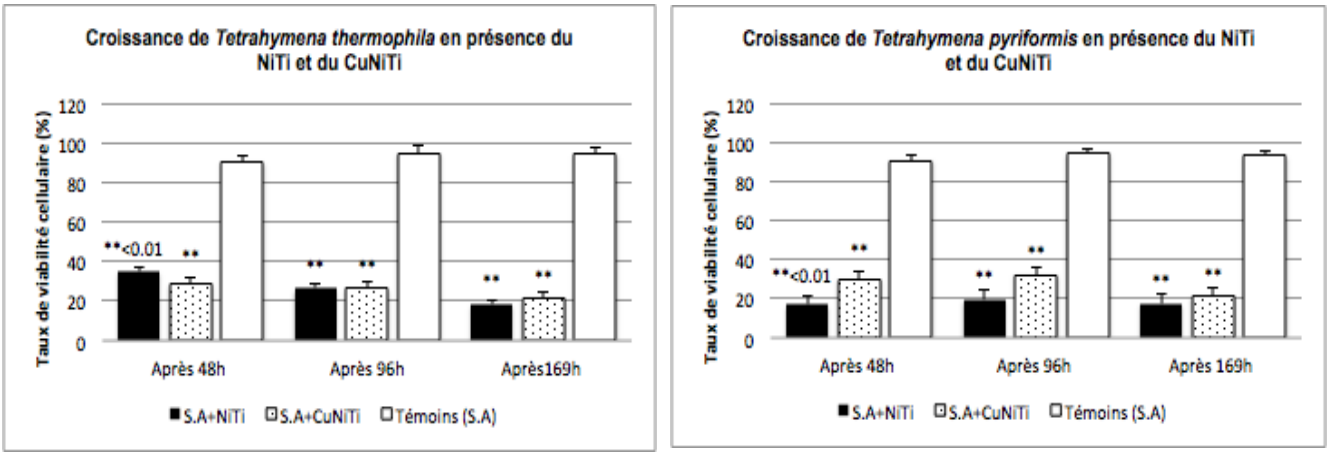

Figure 5: Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of the NiTi and CuNiTi arcs alone. The differences are statistically significant ($p < 0.01$).
S.A: artificial saliva

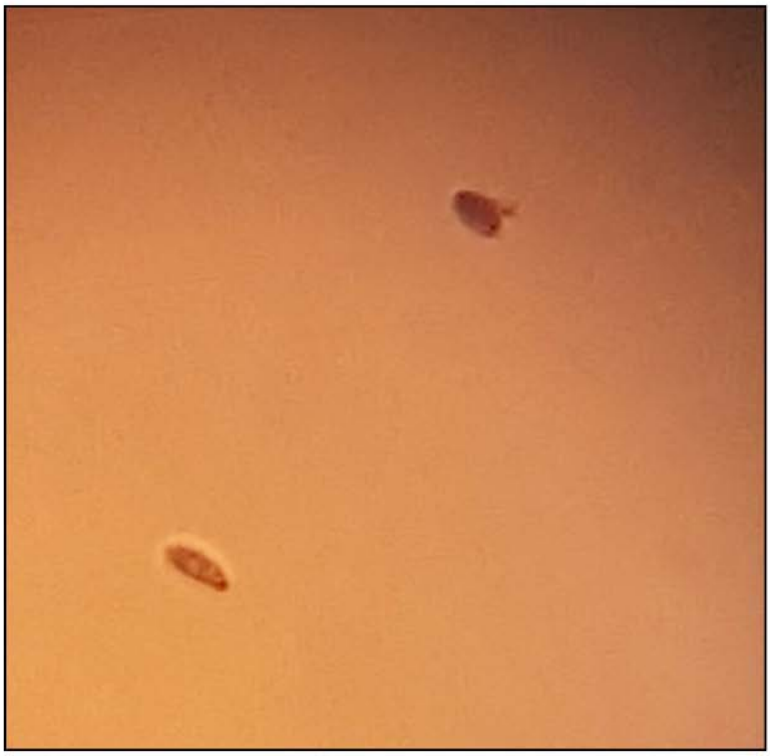

Figure 6: Rounded or elongated forms of Tetrahymena in the presence of orthodontic archwires objectifying their cytotoxicity

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).

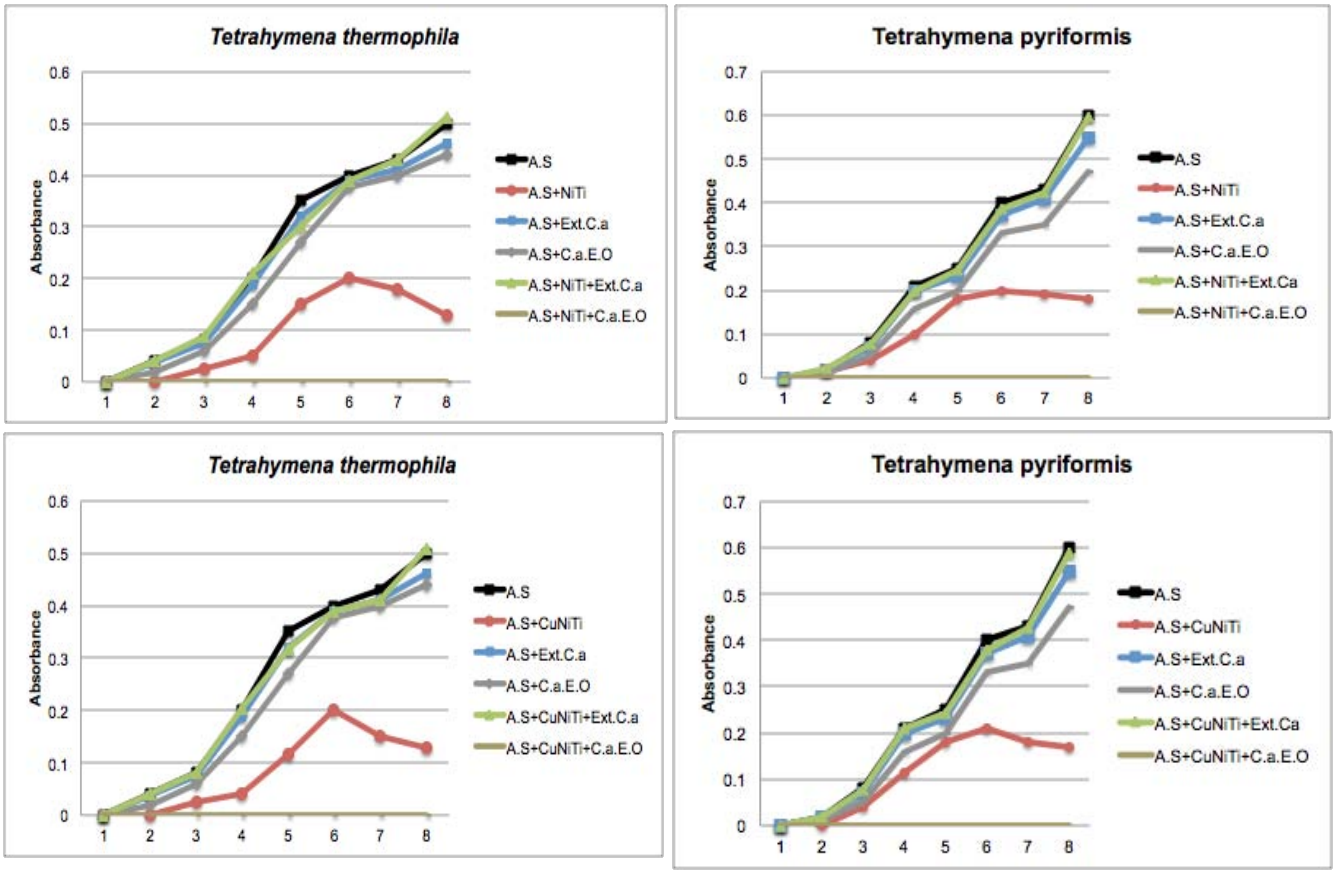

Figure 7: Effect of different types of Celtis australis extracts on the cytotoxicity of NiTi and CuNiTi arcs on Tetrahymena during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva, NiTi: Nickel Titanium, CuNiTi: copper Nickel Titanium, Ext.C.a: Celtis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

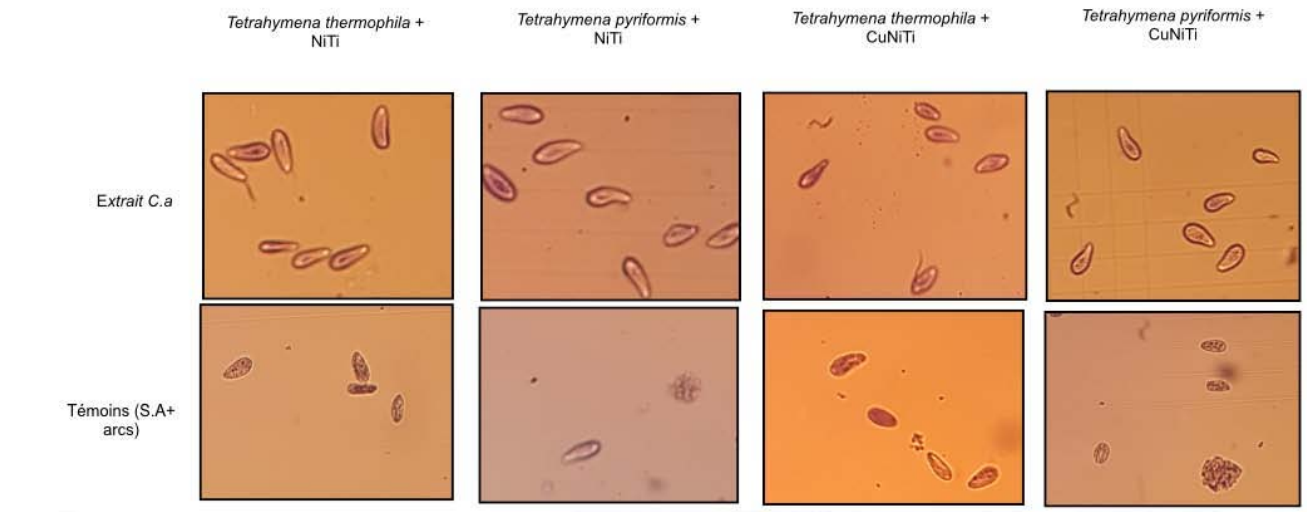

Figure 8: Microscopic images of Tetrahymena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 100 magnification, showing the comparison of their form in artificial saliva and the presence of NiTi and CuNiTi + the extract of Celtis autralis.
C.a: Celis australis, S.A: artificial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

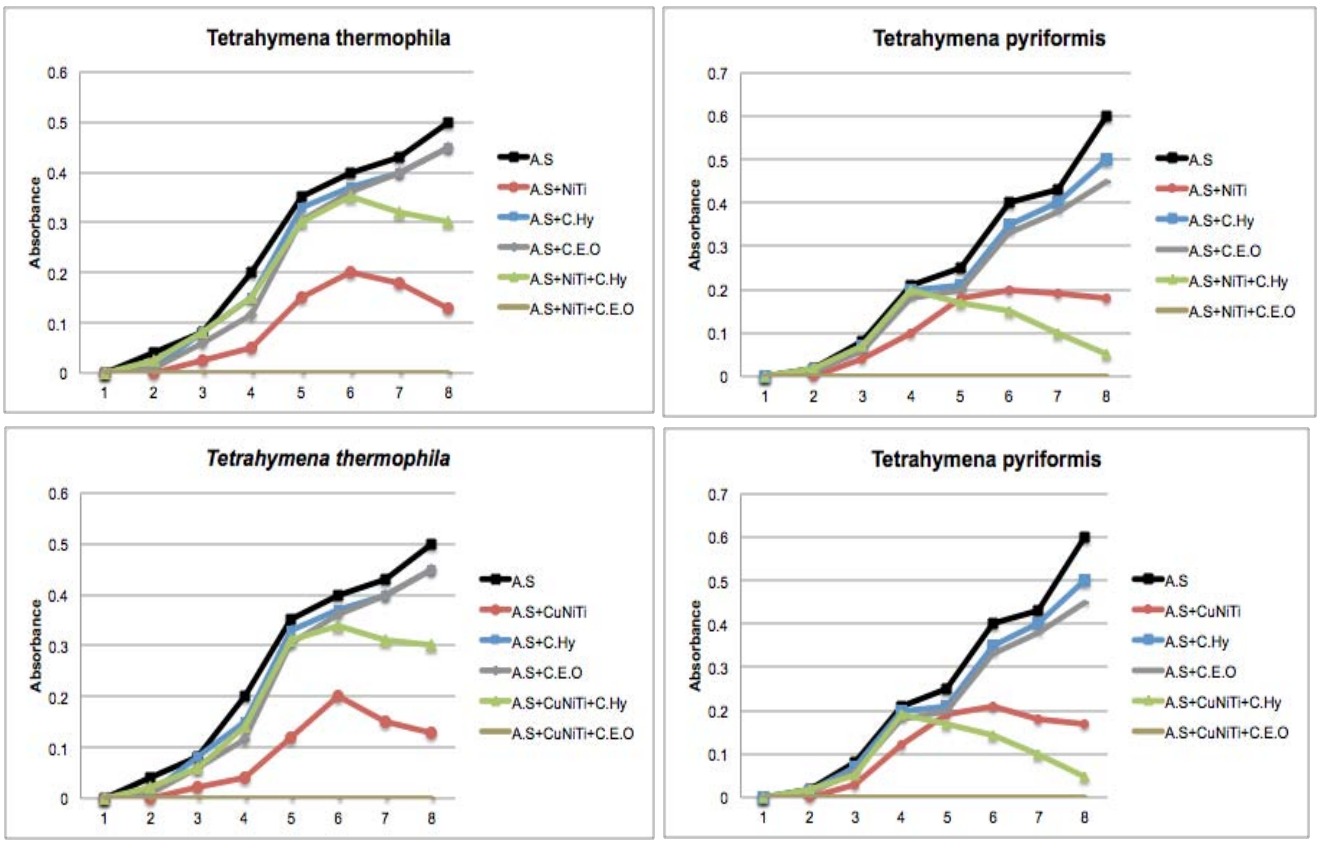

Figure 9: Effect of different types of Syzygium aromaticum (clove) extracts on the cytotoxicity of NiTi and CuNiTi arcs on Tetrahymena during 7 days of culture. Absorbance was determined at 600nm every 24 hours of growth. A.S: Artificial saliva, CuNiTi: copper Nickel Titanium, C.Hy: Clove hydrosol, C.E.O: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

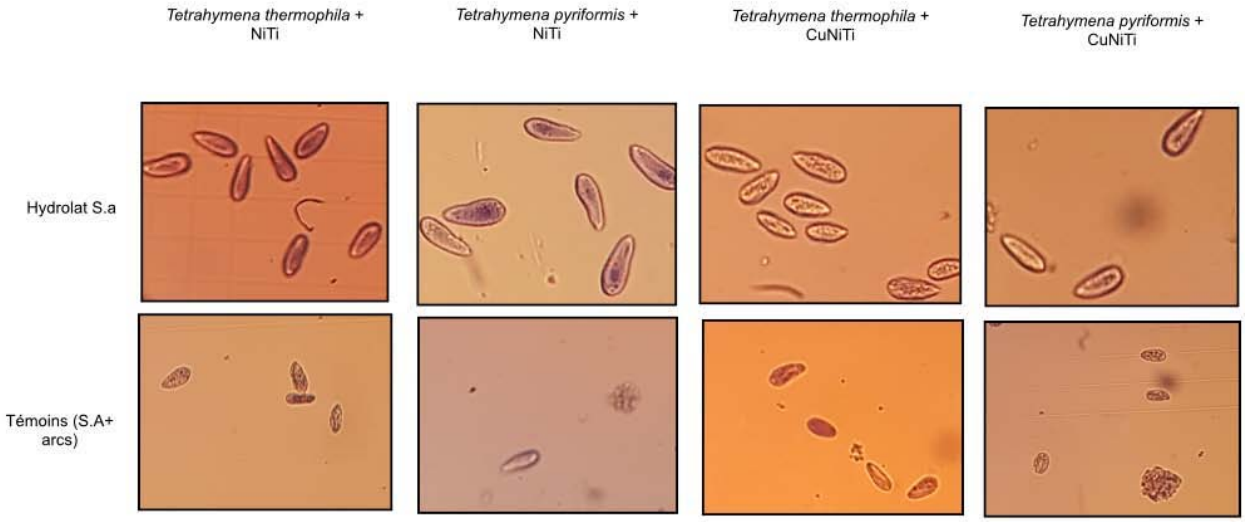

Figure 10: Microscopic images of Tetrahymena thermophila and tetrahymena pyriformis taken after 48 hours of growth at x 10 magnification, showing the comparison of their form in artificial saliva and the presence of NiTi and CuNiTi $^{+}$ the hydrosol of Syzygium aromaticum.
S.a: Syzygium, S.A: artificial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

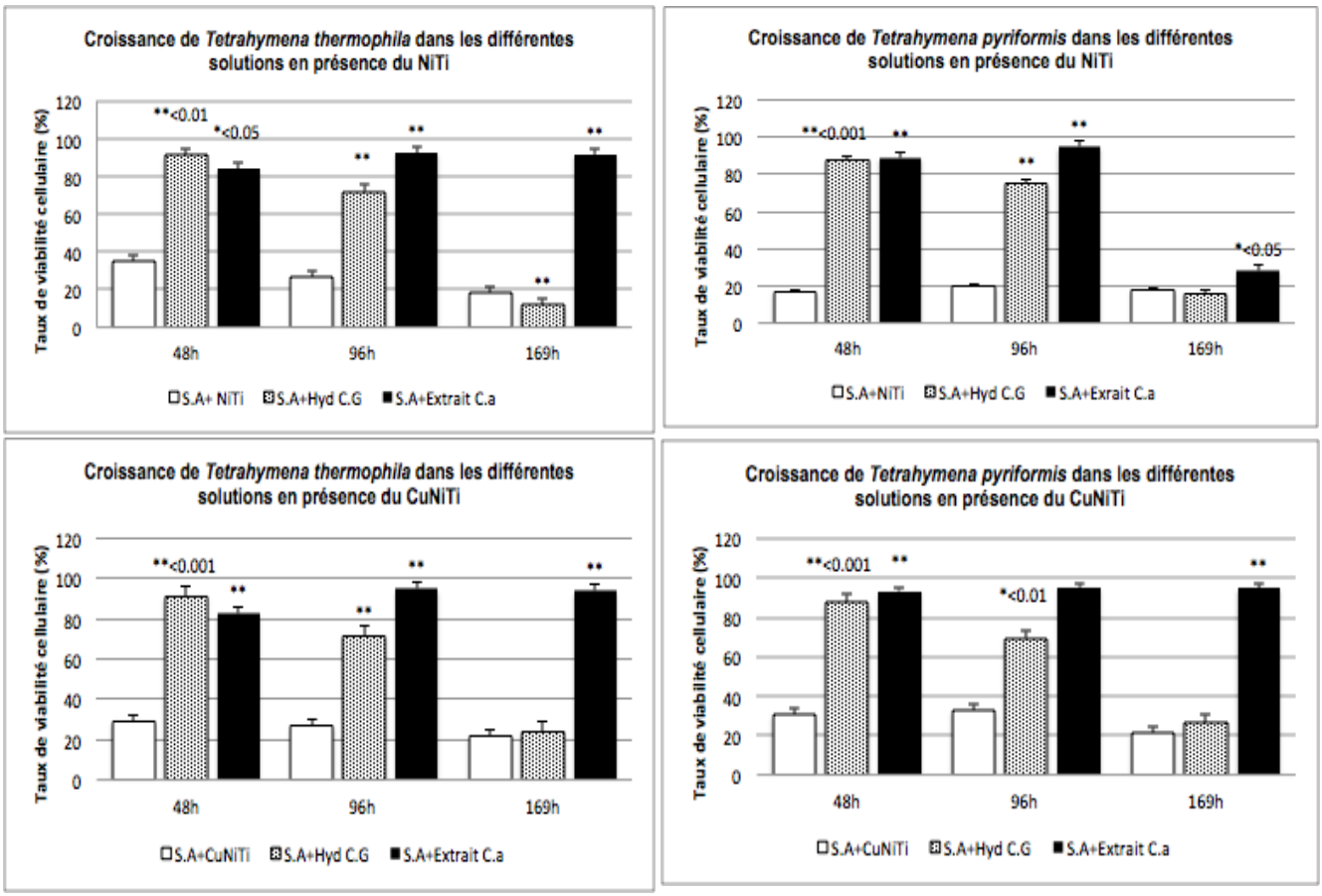

Figure 11: Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of NiTi and CuNiTi arcs and various natural corrosion inhibitors. The differences are statistically significant if $p < 0.05$, very significant if $p < 0.01$ and very very significant if $p < 0.001$.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

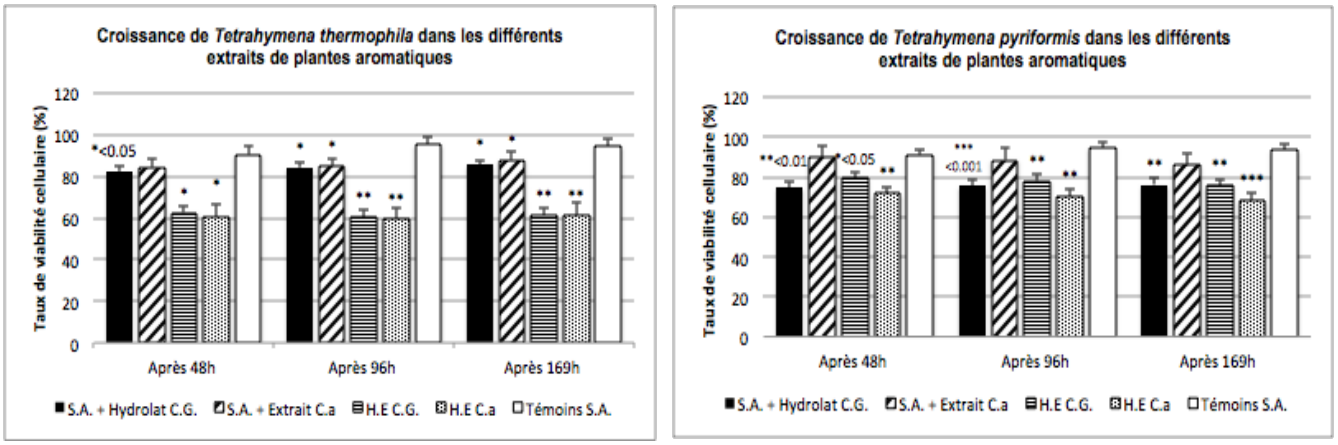

Figure 12: Evolution of the growth of Tetrahymena thermophila and Tetrahymena pyriformis in the presence of the extract, essential oils and the hydrosol alone. The differences are statistically significant if $p < 0.05$, very significant if $p < 0.01$ and very very significant if $p < 0.001$.
S.A: artificial 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.

Citations

1. Brantley WA. Orthodontic wires. In: Brantley WA, Eliades T. Orthodontic Materials: Scientific and Clinical Aspects. Stuttgart, Germany: Thieme; 2000: 78–100.
2. Demele LFM, Cortizo C. Electrochemical behaviour of titanium in fluoride-containing saliva. J Appl Electrochem. 2000; 30:95–100.
3. Dunlap CL, Kirk Vincent S, Barker BF. Allergic reaction to orthodontic wire: report of a case. J Am Dent Assoc. 1989; 118:449–450.
4. Veien NK, Bochhorst E, Hattel T, Laurberg G. Stomatitis or systemically-induced contact-dermatitis. Contact Dermatitis. 1994; 30:210–213.
5. Limberger K. and al., Cytotoxicity of orthodontic materials assessed by survival tests in Saccharomyces cerevisiae, Dental Materials 27 (2011) e81–e86
6. Lewis CG, Sunderman FW. Metal carcinogenesis in total joint arthroplasty. Animal models. ClinOrthop.1996; 329(suppl): S264–S268.
7. Chasapis, C. T. (2018). "Preliminary results from structural systems biology approach in Tetrahyma thermophila reveal novel perspectives for this toxicological model." Archives of Microbiology.
8. Eisen, J.A., Coyne, R.S., Wu, M., Wu, D., Thiagarajan, M., et al., 2006.Macronuclear genome sequence of the ciliate Tetrahymena thermophila, a model eukaryote. PLoSBiol. 4, 1620–1642.
9. Ellison, MTDC, J.C. Madden & T.W. Schultz (Octobre-Novembre 2008). "Definition of the structural domain of the baseline non-polar narcosis model for Tetrahymena pyriformis." SAR and QSAR in Environmental Research 19(N° 7-8): 751-783.
10. Abiola, O. K., Tobun, Y. (2010). Cocos nucifera L. water as green corrosion inhibitor for acid corrosion of aluminium in HCl solution, *Chinese Chemical Letters,* 21. 1449–1452.
11. Ostovari, A., Hoseinieh, S.M., Peikari, M., Shadizadeh, S.R., Hashemi, Corrosion inhibition of mildsteel in 1M HCl solution by henna extract: A comparative study of the inhibition by henna and its constituents (Lawsone, Gallicacid, α-d-Glucose and Tannicacid) S.J., *Corrosion Science,* 51 (2009) 1935–1949. 5.
12. Li, X.H., Deng, S.-D., Fu, H., Inhibition by *Jasminum nudiflorum* Lindl. Leaves extract of the corrosion of cold rolled steel in hydrochloric acid solution *J Appl Electrochem,* 40 (2010) 1641–1649.
13. Saxena, A., Sharma, A., Saxena, D., Jain, P., Corrosion Inhibition and Adsorption Behavior of Clove Oil on Iron in Acidic Medium, *E-Journal of Chemistry,* 9 (2012) 2044-2051.
14. Wataha JC. Principles of biocompatibility for dentalpractitioners. J Prosthet Dent 2001; 86(2): 203–9.
15. Cramer N. B. and al, recent advances and developments in composite dental restorative materials, J Dent Res, 90, 402-16.
16. Eliades Theodore. In vivo aging of orthodontic alloys: Implications for corrosion potential, Nickel release, and biocompatibility. Angle Orthod. 2002; 72 (3): 222-237.
17. Ništiar F., Lukacínová A., Ništiarová A. (2003): Rapid bioassay that uses protozoan Tetrahymena pyriformis to assess the toxicity of various xenobiotics. Lab.Diag. 8: 85-86
18. Benitez L., Martin-Gonzalez S., Gilardi P., Soto T., DeLecea J.R., Gutierrez J.C. (1994): The ciliated protozoa Tetrahymena thermophile as a biosensor to detect mycotoxins. Lett. Appl. Microbiol. 19:489-491C.M.
19. Estrada, E., Uriarte, E., 2001. Quantitative structure–toxicity relationships using tops-mode. 1. Nitrobenzene toxicity to Tetrahymena pyriformis. SAR QSAR Environ. Res. 12, 309–324.
20. HuiLuo, X. L., Tingting Fang, Peng Liu, Chaocan Zhang, HaoXie, Enjie Sun (2015). "The toxicity of binary mixture of Cu (II) ion and phenols on Tetrahymena thermophila." Ecotoxicology and Environmental Safety 113: 412-417.

21. Darcy, P., Kelly, J.P., Leonard, B., Henry, J.A., 2002. The effect of lofepramine and other related agents on the motility of Tetrahymena pyriformis. Toxicol. Lett.128, 207–214.
22. Egloff B. Etudes des salives artificielles utilisées pour les tests de corrosion des alliages orthodontiques. Thèse de deuxième cycle. Université Nancy I. 10/02/2009
23. Zhang Y. and al., Acute Toxicity of Heavy Metals to Tetrahymena in an In Vitro Experiment and Envelope Damage Study, Bull Environ Contam Toxicol (2013) 91:62–68
24. Dayeh R. Vivian and al., Cytotoxicity of metals common in mining effluent to rainbow trout cell lines and to the ciliated protozoan, Tetrahymena thermophila, Toxicology in vitro,19 (2005) 399–410.
25. Ud-Daula A, Gerd P & Karl-Werner S (2013) Method for toxicity test of titanium dioxide nanoparticles in ciliate protozoan Tetrahymena, Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 48:11, 1343-1348.
26. Halliwell B (1994). Free radicals, antioxidants and human diseases: Curiosity, cause and consequences. Lancet 344: 721-724.
27. Nilsson, J.R., 1989. Tetrahymena in cytotoxicology: With special reference to effects of heavy metals and selected drugs. European Journal of Protistology 25, 2–25.
28. Hsiu-Chung O. and al., Protective effects of eugenol against oxidized LDL-induced cytotoxicity and adhesion molecule expression in endothelial cells, Food and Chemical Toxicology 44 (2006) 1485–1495
29. Hammash D. and al., Total phenolic content, flavonoid concentration and antioxidant activity of leaves and bark extracts of Celtis australis L. International Journal of Pharmaceutical Sciences and Nanotechnology, Volume 9 issue 2, March-April 2016.
30. Shokrzadeh M. and al., Antioxidant and protective effect of hydroalcoholic extract of Celtis australis L. on CCl4 induced hepatotoxicity, PharmBiomed Res 2018;4(3): 26-31.
31. Hao Zhu, A. T., Denis Fourches, Alexandre Varnek, Ester Papa, Paola Gramatica, Tomas O ̈berg,| Phuong Dao, ArtemCherkasov, and Igor V. Tetko (2008). "Combinatorial QSAR Modeling of Chemical Toxicants Tested against Tetrahymena pyriformis." J._Chem. Inf. Model.48: 766-784.
32. Rajapakse K., D. D., D. Kastelec, K. Kogej, D. Makovec, C. Gallampois, H. Amelina, G. Danielsson, L. Fanedl, R. Marinsek-Logar & S. Cristobal (2015). "Proteomic analyses of early response of unicellular eukaryotic microorganism Tetrahymena thermophila exposed to TiO2 particles." Nanotoxicology.
33. Fatene N. and al. Assessment of the electrochemical behaviour of Nickel-Titanium-based orthodontic wires: Effect of some natural corrosion inhibitors in comparison with fluoride. J ClinExp Dent. 2019; 11(5):e414-20.
34. Errafiy, N., Ammar, E., Soukri, A., 2013. Protective effect of some essential oils against oxidative and nitrosative stress on Tetrahymena thermophile growth. J.Essent. OilRes. 25, 339–347.
35. Cadi R, Mounaji K, Amraoui F and Soukri A (2013). Protective and antioxidant potential of the argan oil on induced oxidative stress in Tetrahmena pyriformis. Journal of Medicinal Plants Research. Vol. 7 (27), pp 1961-1968.
36. Mar, P.D., et al.Protective effect of oregano and sage essential soils against the effect of extracellular H2O2 and SNP in *Tetrahymena thermophila* and *Tetrahymena pyriformis* Journal of King Saud University– Science, volume 32, issue 1, January 2020, pages 279-287.
37. Abbasitabar F (2017). "In silico prediction of toxicity of phenols to Tetrahymena pyriformis by using genetic algorithm and decision tree-based modeling approach." Chemosphere 172: 249-259.
38. Govind Sharan G. and al. (2017). "Hetero agglomeration of zinc oxide nanoparticles with clay mineral modulates the bioavailability and toxicity of nanoparticle in Tetrahymena pyriformis." Journal of Colloid and Interface Science 495: 9-18.