Assessment of orthodontic biomaterials’ cytotoxicity: an in vitro study on cell culture

ALINA SODOR¹, ALEXANDRU SIMION OGODESCU², TUDOR PETREUȘ³, ALINA MARIA ȘIŞU⁴, IRINA NICOLETA ZETU⁵

¹Department of Orthodontics, Faculty of Dental Medicine, “Grigore T. Popa” University of Medicine and Pharmacy, Iassy, Romania
²Department of Pedodontics–Orthodontics, Faculty of Dental Medicine, “Victor Babeș” University of Medicine and Pharmacy, Timisoara, Romania
³Department of Cell and Molecular Biology, “Grigore T. Popa” University of Medicine and Pharmacy, Iassy, Romania
⁴Department of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, Timisoara, Romania

Abstract
Objective: Orthodontists use various biomaterials such as molar bands, brackets, archwires, transpalatal archwires, facial masks and other auxiliary devices. One of the essential properties of these materials should be the biocompatibility. The aim of this study was to evaluate the biocompatibility of some orthodontic biomaterials like stainless steel archwires, brackets and NiTi (nickel–titanium alloy) coil springs. Materials and Methods: The studies were performed in vitro using human fibroblasts cultures on which the orthodontic materials were applied. The positive control was the copper amalgam. Readings of the cell reactions were performed at three and six days. Results: It was observed that the materials used in the study cause cell alterations of variable intensity. The metallic brackets represent an important cell stress factor causing shape changes. For the metallic archwires, a preferential tropism for different areas of the bracket was also obvious. We observed a preferential tropism for the areas between the NiTi coil spring spirals. For the stainless steel archwires, we observed at six days a decay of cell density and also a higher amount of cells near the archwire areas on which bends were performed. Conclusions: All biomaterials analyzed in our study cause cellular changes of varying intensity without necessarily showing a cytotoxic character.

Keywords: orthodontic biomaterials, cytotoxicity, biocompatibility.

Introduction
Since Edward Angle’s time until now, orthodontics has showed a great progress in what concerns techniques and materials.

At the beginning of the 20th century, the materials used in orthodontics were gold, platinum, ivory, zinc, copper and vulcanite. Nowadays orthodontists use various biomaterials of great complexity. The metallic biomaterials must satisfy requests of resistance, stability, elasticity and nevertheless biocompatibility. During the orthodontic treatment, the oral tissues interact with several metallic devices like molar bands, brackets, archwires, transpalatal archwires, and other auxiliary devices. The metal alloys that are used to obtain orthodontic biomaterials have a complex composition of the same material, sometimes more than one structure being present [1].

Usually, the most commonly used archwires are: the stainless steel archwires, the β-titanium archwires, the cobalt–chrome–nickel archwires, and nickel–titanium archwires [2].

In the different stages of treatment, the orthodontist chooses the archwires according to their degree of deformation, elasticity, frictional properties and last but not least according to the orthodontic forces and the way of producing the orthodontic force [3].

The metal brackets raise problems concerning their resistance and biocompatibility. There are many studies that aim to evaluate the corrosion of metallic orthodontic devices, corrosion responsible for allergic reactions, and the decrease of their properties.

Regarding the biocompatibility, there are studies that prove the corrosion of the AISI type 316L alloy used to produce metallic brackets. The L specification points the low carbon content of the alloy that contains at most 16–18% Cr, 10–14% Ni, 2–3% Mo and 0.03% C. Although studies prove a good behavior of this alloy, there is a degree of corrosion shown by the discoloration of the adhesive layer [3].

Kocadereli et al. analyzed the chromium and nickel concentration in the saliva of the patients treated with orthodontic fixed devices, observing that these devices do not change significantly the saliva composition at two months after the treatment starts [4].

Ağaoglu et al. evaluated the saliva and serum content of nickel and chromium during the entire orthodontic treatment. The study pointed out important differences of nickel and chromium content in different stages of the treatment. During the second year of treatment, a significant rise in the ionic levels was observed in the serum. In the saliva, a maximum level of these ions was reached during the first month of treatment. The authors concluded that following the orthodontic treatment there are significant changes of the serum and saliva nickel and chromium content without reaching a toxic level [5].

In orthodontics, the main allergens are the metal salts from the metallic orthodontic devices, monomers, cross-
linking agents, and chemical substances associated to the polymerization, the latex from the gloves and substances mixtures [2].

There is no unanimous opinion concerning the allergic reactions due to the substance found in the composition of orthodontic devices and materials as well as the level of ion release with harmful potential in the oral environment.

Resin-based composites are cytotoxic before polymerization and immediately thereafter, whereas already set specimens cause almost no reaction [6].

An attempt of assessing the prevalence of allergic reactions in the 90s estimates that one of 100 individuals develops allergic reactions. The reactions observed may have included irritative reactions and hypersensitivity [7].

The aim of this study was to evaluate the biocompatibility of some orthodontic biomaterials like stainless steel archwires, brackets and NiTi coil springs.

Materials and Methods

Three groups of five materials were made, and were considered the study group. The materials were sterilized for 60 minutes with an autoclave. For a better contact with the cells, the archwire was bent into a helix shape.

The positive control group was represented by fragments of copper amalgam.

The negative control group was represented by cells not exposed to any material.

The cells used in our experiment were NHDF cells (normal human dermal fibroblasts) in a proliferative stage, with a normal subcultivated density of 3500 cells/cm². The monolayer confluence for subcultures was obtained in nine days. The cells obtained certificates of viability and survival before the delivery. After the fibroblast reached 90% confluence the recipients were three trypsinized with 2 mL Trypsin EDTA and then washed by centrifugation at 300×g for 5 minutes, after which they were re-suspended in 40 mL Dulbecco’s medium improved with 0.584 g/L L-Glutamine, 4500 mg/L Glucose, 10% FBS (fetal bovine serum), 1% antibiotics – Penicillin/Streptomycin (0.06 mg/mL Penicillin and 0.1 mg/mL Streptomycin sulfate), 1% Amphotericin B. The medium containing the cells was agitated and divided in four flasks. In these mediums, the cells were left until they reached 90% confluence (seven days). After seven days, all recipients were trypsinized, washed by means of centrifugation at 300×g for 5 minutes and after that suspended in 1 mL medium, and counted. Then the cells were divided into three groups and placed on three trays with 24 wells.

In the first eprouvette of each tray, the negative control group was placed in the same improved culture medium and in the second well the positive control material. In the following eprouvettes, the following materials were placed: NiTi coil springs (Group A), metallic brackets (Group B) and orthodontic stainless steel archwires (Group C).

Following that, the trays were incubated at 37°C and 5% CO₂. Direct microscopic reading was performed at three and six days. The cells were photographed at the times of the reading using a phase contrast microscope, Nikon Eclipse TE300 and its software. The photographs were made with different objectives/lens 4×, 10× and 20×. The presence and shape of the fibroblasts around the material were analyzed, as well as the shape and the number of those at a distance.

Results

After three days, the negative control group was populated with fibroblasts with a confluence of 70–80% (Figure 1).

For the positive control group, we observed apoptosis of all the cells.

For Group A, after three days, the presence of normal fibroblasts was noticed, which adhere well in the proximity of the applied material (Figure 2a). The aspect of fibroblasts situated at a distance from the material is also normal, with rounding of the cell body and a normal cell density compared with the control group (Figure 2b). Thus, it may be appreciated that after three days this material is not toxic to the cells in the culture.

For Group B, after three days, the presence of fibroblasts adhering well in the proximity of the applied material was noticed (Figure 3a). Some of the cells have a globular body, inflated, suggesting cell damage. Further away from the material, the aspect of fibroblasts is normal, the number of cells with rounded cell body being very important. The number of cells with shape changes is higher compared to that in Figure 1. The cell density away from the material is moderately reduced compared to the control group (Figure 3b). After three days of cultivation, it is considered that the material is a stress factor at the cellular level for the cells in culture. Normally, grown cell distribution is not uniform in the different regions of the material. Thus, in the corners (Figure 3c), there is a lower cell density than in the rectilinear regions (Figure 3d).

In Group C, after three days, the presence of normal fibroblasts was noticed, well adherent in the proximity of the applied material (Figure 4a), but with marked differences between the outside part of the metallic material and the spaces between the coils obtained through bending the archwire. It was noted that increased cell density was present between the coils; the multiplied cells show a pronounced tropism for metal coils (Figure 4, b and c). In Figure 4a, fibroblasts proliferate more clearly
around the free end of the coil. In-between the coils of the material, some cells are pyknotic or with swelling body, these being signs of moderate cell suffering. Further away from the material, the aspect of the fibroblasts is normal, but their number is small and the cell density farther from the material is moderate compared to the control group. After three days of cultivation, you can observe the good compatibility of the material, toxicity seeming to be absent. Distribution of normally grown cells differs in-between the coils of the material and at its periphery (Figure 4d).

Results six days after the application: Materials were left in contact with cells only six days because the rate of multiplication of fibroblasts (Lonza) was increased by adding culture medium with specific growth factors.

At six days, the well with the negative control was populated by fibroblasts, with a confluence of about 90–95% (Figure 5).

![Figure 2](image1.png)

**Figure 2** – Fibroblasts, three days after application (×40): (a) In contact with the NiTi material; (b) Away from the material.

![Figure 3](image2.png)

**Figure 3** – Fibroblasts, three days after application (×40): (a) In contact with the bracket, mixed area; (b) Away from the material; (c) In contact with the corner of the bracket; (d) In contact with the straight bracket area.
For Group A, six days after application, the presence of normal fibroblasts was noticed, well adherent to the proximity of the material applied (Figure 6a). The aspect of the fibroblasts away from the material is also normal, with rounding of the cell body and normal cell density compared to the control (Figure 6b). Compared to the three-day aspect, no increase in cell density can be noted. Furthermore, cells in the proximity of the coil spring start to exhibit shape alterations. Cell density between the coils is reduced. After six days, this material might not be toxic for the cells but induce some changes that may be related to the formation of oxide at the material–tissue interface.
For Group B, six days after application, we noticed the presence of normal fibroblasts, well adherent to the proximity of the material applied. The normally grown cell distribution is not uniform in the different regions of the material or relief of the bracket (Figure 7a). Normal cellular appearance and cell density in contact with the material varies: away from the material it is increased, similar to the control group, in contact with the material, near the bracket hook, it is reduced (Figure 7b). At six days, this material could be a stress factor at cellular level for the cells in culture, but only in certain areas.

In Group C, six days after application, we noticed the reduced fibroblast presence, which adheres to the well in the proximity of the applied material (Figure 8a), but with marked differences between the outside of the material and the spaces between the coils. Increased cell density is still present between the coils, the multiplied cells showing a pronounced tropism for metal coils (Figure 8b). Away from the material, the fibroblasts aspect is normal, but their number remains low. The cell density away from the material is moderately reduced compared to the control group (Figure 8c). At six days of cultivation, this material has good compatibility and toxicity seemed to be absent. Normally grown cell distribution differs between the periphery of the material and its coils (Figure 8b).

**Discussion**

Developments in technology and treatment methods marketed a wide variety of biomaterials that must be above all biocompatible.

The metal orthodontic biomaterials often pose problems of cytotoxicity due to corrosion products released.

Due to the corrosion processes, ions with potentially cytotoxic activity like nickel may be released into the oral cavity.

Mockers et al. in 2002 and Montanero et al. in 2006 studied the nickel biocompatibility issues [8].

Oller et al. have demonstrated the carcinogenic potential of nickel and Faccioni et al. have shown in their studies that the nickel ion is genotoxic [9, 10].

There have been studies that show that nickel ion is mutagenic [11].

Grimsdottir et al. studied the cytotoxicity of orthodontic metallic biomaterials, and no cytotoxic effects were observed for orthodontic springs but show a cytotoxic effect in multicomponent devices [12]. The authors advocate a higher cytotoxicity of copper in these devices than nickel, explaining the most pronounced cytotoxic nature of the devices upon which welding was performed using welding copper alloys compared to those unwelded.

Similar results obtained Mockers et al. [8].

There have also been studies that have shown biocompatibility of NiTi alloys, their tissue acceptability being similar to that of other medical alloys [13, 14].

In our study, the conditions were the same for each material. Sample handling and reading results occurred in similar circumstances, and to ensure accurate results, microscopic analysis was performed by the same assessor.

Our research was conducted in vitro, according to ISI (Indian Standards Institution) 1999 specifications.

Eliades et al. in 2004 showed that the corrosion resistance of metallic biomaterials depends on oral environmental factors such as quantity and quality of saliva, pH beverages and foods consumed [15].

Although the environment in which our experiments were made does not reproduce the complexity of the conditions of the oral cavity, we obtained data that remains useful to determine the cytotoxicity of metallic biomaterials.

Our results show a good biocompatibility of NiTi
coil spring at three and six days even though it causes cell shape changes, which might be due to the release of corrosion products. We also observed a more pronounced cell tropism for the space between springs.

Bogdanski et al. also observed shape cell changes in contact with NiTi-based biomaterials and states that NiTi-based biomaterials are biocompatible if the two metals are in 50:50 percent [16].

Sadeghian et al. demonstrated their cytotoxicity after subdermal implantation of nickel titanium springs samples [17].

For the metal brackets, we observed a difference in cell tropism for different areas of the bracket with a smaller presence on the bracket wing, possibly due to either the release of corrosion products from the solder that was used to weld it, or from some composition variations of the alloy in the area.

Costa et al. assessed in vitro cytotoxicity of metal brackets observing higher cytotoxicity of the stainless steel brackets [18].

Rusu et al. revealed the non-cytotoxicity of the Ni–Cr alloy and the Co–Cr alloy that can be used successfully in dental practice, despite the tendency to give up metal in this medical field [19].

Besides, the biomaterial application resulted in significant changes in cell shape, which betrays a cellular stress factor, also probably due to release of metal ions. After six days cell proliferation is not observed, which correlates well with the harmful effects of the material.

Based on similar observations, it is speculated in literature that corrosion products of orthodontic biomaterials released from the metal can cause localized gingivitis seen in some patients treated with fixed orthodontic appliances [12].

Regarding the orthodontic stainless steel springs analyzed, after three days we observed normal cell density with cell tropism for coil areas simulating the arch bends made during treatment. However, at six days there is a decrease in cell density in both proximity and further away from the material.

Kao et al., studying the biocompatibility of four representative types of brackets applied on different types of cells, found that although these biomaterials have been shown to be biocompatible, they cause different cellular responses [20].

Applying orthodontic spring fragments in cultured cortical cells, David & Lobner noted that the NiTi orthodontic springs and titanium-molybdenum are not neurotoxic, while the stainless steel and Elgiloy have a pronounced toxic character [21].

Our results provide an overview of the biocompatibility of metallic biomaterials widely used in practice. The analysis performed under the same conditions of some biomaterials often used simultaneously in orthodontic practice allows the estimation of local effects that may result from treatment due to their individual and summed up influence. It can also explain local phenomena that occur at different stages of the orthodontic treatment, for example for space creation when brackets, stainless steel archwires and coil springs are used.

Conclusions

All biomaterials analyzed in our study cause cellular changes of varying intensity without necessarily showing a cytotoxic character. Metal brackets are important cellular stress factors, causing various changes. For metal brackets, cells have different tropism for different parts of the bracket. The cells have a preferential tropism for the areas between the coils of NiTi coil spring. There is a decrease in cell density at six days for the stainless steel archwires. It was observed an increase in cell density in the archwire areas on which bendings were practiced.

Conflict of interests

The authors declare that they have no conflict of interests.

References

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Corresponding author
Alexandru Simion Ogodescu, Lecturer, MD, PhD, Department of Pedodontics–Orthodontics, Faculty of Dental Medicine, “Victor Babeș” University of Medicine and Pharmacy, 9 Revoluției Avenue, 300041 Timișoara, Romania; Phone +40723–544 336, e-mail: ogodescu@yahoo.com

Received: February 6, 2015

Accepted: October 18, 2015