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THE CYTOTOXICITY OF ORTHODONTIC POLYMERIC BIOMATERIALS

ALINA SODOR, GEORGETA ZEGAN*, LOREDANA GOLOVCENCU*, DANIELA ANISTOROAEI

University of Medicine and Farmacy Grigore T. Popa, Department of Orthodontics, 16 Universitatii Str., 700115, Iasi, Romania
*Corresponding author: Georgeta Zegan, e-mail: georgeta.zegan@umfiasi.ro
*Corresponding author: Loredana Golovcencu, e-mail: loredana.golovcencu@umfiasi.ro

ABSTRACT The objective of our study was to evaluate in vitro the cytotoxicity of different intraoral elastic chains and elastomeric ligatures that are used as components of orthodontic fixed appliances. The study evaluated the reaction of tree groups: the negative control group (human fibroblasts cultures), the positive control group (dental copper amalgam) and the study group represented by intraoral orthodontic elastics: elastic chain (Orthoclassic) and elastomeric ligatures of different colors and latex consistency from different manufacturers (Orthoclassic and GAC). After 3 and respective 6 days we analyzed the samples by direct method and microscopic analysis (Nikon Eclipse TE300). We analyzed the shape of the fibroblasts around the material, their number and the morphologic alterations of the cells. The analysis of the fibroblasts situated at distance from the material and the percent of the vital cells was obtained by comparing the maxim cells density of the negative control group with the density of the study groups. The dental copper amalgam determined the apoptosis of all the cells of the group and showed a high cytotoxicity. The elastic chain Orhoclassic determined important morphologic changes on human fibroblasts cultures and showed a reduced biocompatibility. The elastomeric ligatures Orthoclassic with low elasticity showed a relative biocompatibility. The elastomeric ligatures Orthoclassic with high elasticity showed a good biocompatibility. The elastomeric ligatures GAC showed a relative compatibility at 3 days but a high cytotoxicity at 6 days.

Key words: dental copper amalgam, elastic chain, elastomeric ligatures, cytotoxicity

Orthodontists use a wide area of biomaterials that must be the less harmful possible. Although the problems raised by the orthodontic biomaterials are similar to those raised by the dental ones they need a different approach.

During the orthodontic treatment, the tissues are subjected to contact with appliances, which establish a large contact area with the oral mucosa. An adverse reaction following contact with the orthodontic appliances is the allergy. The potential allergens in orthodontics that are part of the orthodontic appliances are metals, monomers, cross-linking agents, polymerization chemicals, latex related components and miscellaneous factors [1]. The elastomeric ligatures and elastic chains are polyurethanes [1]. The orthodontic material companies have different technological approaches, in order to obtain the polyurethane products. The two main methods of processing the elastomeric modules are injection molding and die stamping.

The first latex allergy cases were reported at the early ’80 in Europe [2]. It’s incidence is still unknown, although there were developed tests in order to asses it’s frequency [3]. The orthodontic companies tried to minimize the allergen agents in latex products. The most common allergens in latex products are Hev b0.02 and Hev b 5. Other two common allergens determined

allergies on children with spina bifida, Hev b 3 and Hev b 1 [4]. Children with spina bifida have shown the highest prevalence of latex allergy [3]. The severity of the allergy varies from skin rash, oral lesions, and the most severe the anaphylactic reactions [4]. The prevalence of severe reactions has increased in the last years [5].

In order to asses the harmful potential of substances one method is to evaluate their cytotoxicity. The cytotoxicity is the capacity of one substance to be harmful to the cells, consecutive to the cells reaction.

Experimental part

The objective of our study was to evaluate in vitro the cytotoxicity of different intraoral elastomeric ligatures and elastic chains that are used as components of orthodontic fixed appliances.

The study evaluated the reaction of tree groups: the negative control group, the positive control group and the study group. As negative control group we used human fibroblasts cultures (Normal Human Dermal Fibroblasts - NHDF). As positive control group we used dental copper amalgam (Amalgam Alloys (Pty) Ltd). For the study group we used elastic chain (Orthoclassic) and elastomeric ligatures of different colors and latex consistency from different manufacturers (Orthoclassic and GAC).

The experiment was performed on NHDF. Cells were acquired in subcultivated, proliferative stage and have been assessed prior the transportation by means of viability. The cells were transported in hermetically closed recipients, on the temperature condition required by the provider. In the laboratory, after 9 days it was obtained the monolayer confluence for cultivation. Subsequently, the cells were trypsinized, washed and suspended in Dulbecco supplemented medium.

The fragments of 1 cm elastic chain and elastomeric ligatures were sterilized prior the experiment for 30 minutes with an autoclave, according to the manufacturer specifications.

In order to eliminate reading errors and to obtain more accurate results, the experiment was conducted on three trays, with 24 wells each, in which we was applied an equal number of cells. For each plate the following protocol was performed: the first wells of each tray was the negative control group, on the second well it was added dental copper amalgam fragments representing the positive control group, on the other 22 wells there were applied the samples of materials that formed the study group. The trays were incubated at 37°C and 5% CO_2. After incubating of the samples, microscopic analyses were performed at three and six days by 4x, 10x and 20x magnification. The examination was performed with a Nikon phase-contrast microscope Eclipse TE300. Photographs of the wells were taken.

It was performed a morphological analysis of the cells situated in the proximity and at distance from the material and also an analysis of their density.

Results and discussions

The 3 days analysis on the negative control group reveals a fibroblast population with a confluence of 70-80% (Fig.1a). On the positive control group no vital cells were observed.

For elastic chain (Orthoclassic) after 3 days we observed the presence of normal fibroblasts, adherents to the well in the proximity of the material (Fig.1b). There were also noticed high differences on the fibroblasts inside the elastic chain eyelets and outside of them. Inside the elastic chain eyelets, the cells seem to have a normal multiplication rate and a morphologic aspect

similar to the ones in the negative control group. The cells grown outside the elastic chain suffered great morphologic changes, therefore most of them were picnotic, others had an enlarge bogy, suggesting a major cell damage. The aspect of the cells situated at distance from the material was modified; the number of the cells with rounded cell body was significant, being almost equal to the normal ones. The cell density at distance from the material was highly reduced, comparative with the negative control group (Fig. 1c). The normal cells distribution was different inside and outside the elastic chain. Outside the elastic chain and next to it (Fig. 1d-e), the number of cells with normal growth is smaller than the one of the inside chain. Under the material, the cells suffered major changes and we observed picnosis and apoptosis.

Fig. 1. Fibroblasts of the negative control group (a) and in contact with the elastic chain (b-e) after 3 days

The elastomeric ligatures Orthoclassic with low elasticity, at 3 days analysis showed the presence of normal fibroblasts, adherents to the well in the proximity of the material (Fig. 2a). Another important aspect is the significantly decreased cell density close to the material (Fig.2a-b). The aspect of the fibroblasts situated at distance from the material was normal, but their number was reduced. The cell density at distance was moderate, compared to the negative control group (Fig. 2c). It may be considered that 3 days of cultivation this material exhibits a relative compatibility. The normal cells distribution differs from its proximity to the periphery (Fig. 2d).

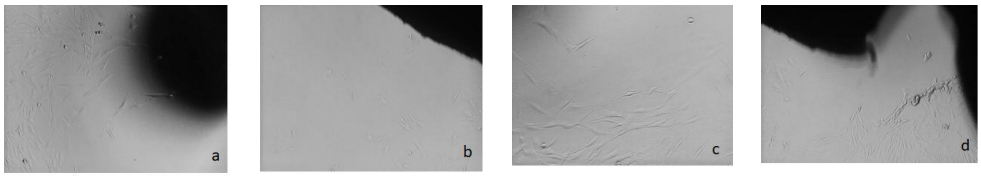

Fig. 2. Fibroblasts in contact elastomeric ligatures Orthoclassic with low latex consistency after 3 days

For the elastomeric ligatures Orthoclassic with high elasticity after 3 days we observed the presence of normal fibroblasts, adherent in the proximity of the material (Fig. 3a), without significant differences compared to the negative control group. The cell density was higher close to the material (Fig. 3a-c), than at distance from it (Fig. 3d). The aspect of the fibroblasts at distance from the material was normal, and their number was moderate. It can be appreciate that at 3 days after cultivation this material exhibits good compatibility, and the toxicity seems to be absent.

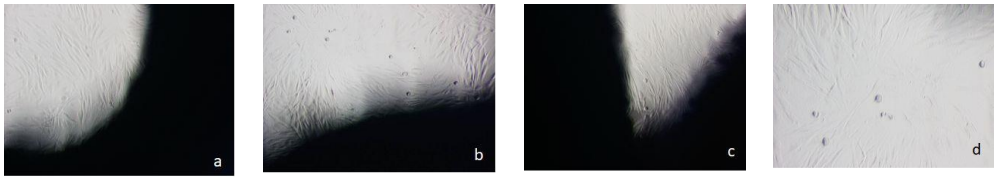

Fig. 3. Fibroblasts in contact elastomeric ligatures Orthoclassic with high latex consistency after 3 days

For the elastomeric ligatures GAC, at 3 days it was noticed the presence of normal fibroblasts, adherent to the well in the proximity of the material (Fig. 4a). The cell density is smaller in the proximity of the material (Fig. 4a-b) than at distance from it (Fig. 4d). The aspect of the fibroblasts at distance from the material is normal, but their number is moderate to low. The cell density at distance from the material is moderate, compared to the negative control group. We can appreciate that 3 days after cultivation, this material exhibits a moderate compatibility, and the toxicity seems to be reduced. The results are similar to those obtained for the elastomeric ligatures Orthoclassic with high elasticity.

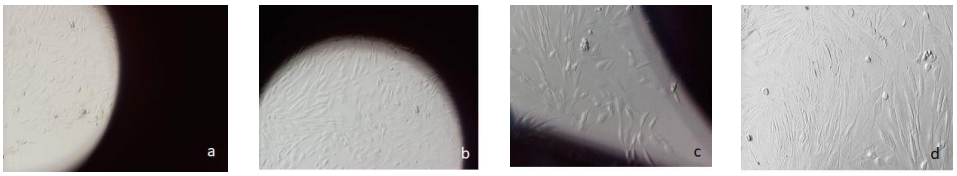

Fig. 4. Fibroblasts in contact elastomeric ligatures GAC after 3 days

Another evaluation was performed at 6 days, because the multiplication rate of the fibroblasts was increased by adding the suitable growth factors. At 6 days, the negative control group had a confluence of 90-95% (Fig. 5a). For the positive control group no vital cell was observed.

For the sample A after 6 days it was observed the presence of normal fibroblasts in the proximity of the material, and also important differences between the fibroblasts inside the elastic chain and outside it (Fig.5b). The cell density was superior to the one observed at 3 days, but lower then the one of the negative control group. Inside the elastic chain the cell density was lower, but the morphological aspect of the cells is comparable to the normal ones. Under the elastic chain there were no viable cells. The aspect of the fibroblasts at distance from the material was modified; the number of cells with rounded body was increased, being almost equal to the number of the normal cells. The cell density at distance from the material was reduced (Fig. 5c). It can thus be appreciated that the 6 days the material represents a cellular stress factor. The distribution of cells differs inside the eyelet and outside the material. Outside the material and in its proximity (Fig. 5d-e), the number of cells is lower than the one of cells grown in the eyelets of the chain.

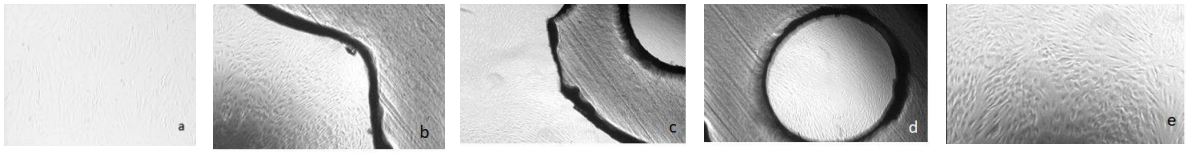

Fig. 5. Fibroblasts of the negative control group (a) and in contact with the elastic chain (b-e) after 6 days

The elastomeric ligatures Orthoclassic with low elasticity analysis at 6 days shows the presence of some normal fibroblasts, adherent to the well in the vicinity of the applied material (Fig. 6a). The cell density is markedly reduced in the proximity of the material (Fig. 6b-c). The aspect of the fibroblasts at distance from the material is normal, but their number is still reduced. The cell density at distance from the material is moderate, compared to the negative control group (Fig. 6d). It can be appreciate that at 6 days this material exhibits a relative biocompatibility.

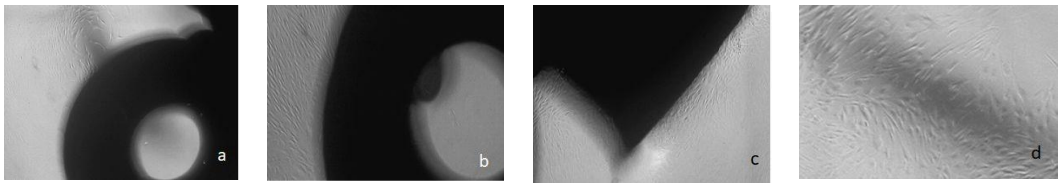

Fig. 6. Fibroblasts in contact elastomeric ligatures Orthoclassic with low latex consistency after 6 days

The elastomeric ligatures Orthoclassic with high elasticity analysis at 6 days shows the presence of normal fibroblasts in the proximity of the material (Fig. 7a). The cell density is still higher in the proximity of the material (Fig. 7b) than at distance (Fig. 7c). The cell density at distance from the material is moderate compared to the negative control group (Fig. 7d). We can appreciate that at 6 days this material exhibits good compatibility, and toxicity seems to be absent.

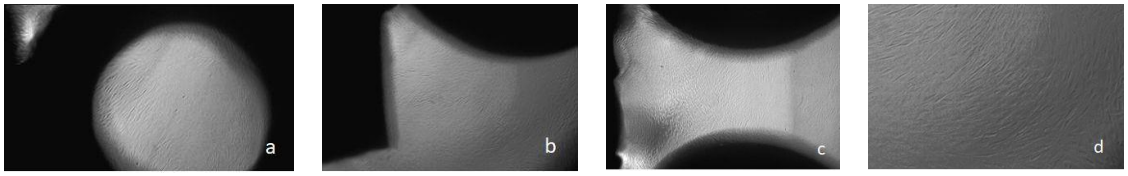

Fig. 7. Fibroblasts in contact elastomeric ligatures Orthoclassic with high latex consistency after 6 days

The elastomeric ligatures GAC at 6 days are characterized by the disappearance of normal fibroblasts, adhering to the well in the vicinity of the applied material (Fig. 8a). Cells disappeared completely around the material, but keep a normal aspect at distance (Fig. 8b-c). It can be thus being appreciated that the 6 days the material is toxic to the cells

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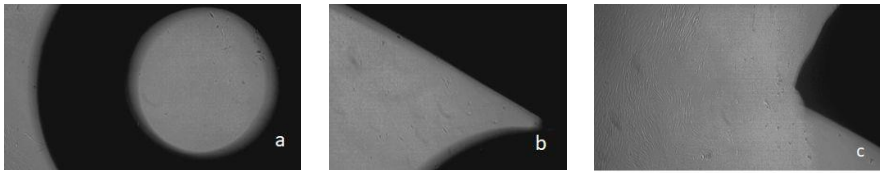

Fig. 8. Fibroblasts in contact elastomeric ligatures GAC after 6 days

In the last decades, due to the high development of the biomaterials, their appropriate selection is a big challenge. The biological answer caused by the biomaterials may represent by toxic, inflammatory, allergic or mutagenic reactions. The evaluation of the cytotoxicity is one of the fundamental parameters in order to asses the biologic response [6].

The biomaterials used during the orthodontic treatment are in permanent contact with the oral fluids and also subjected to their actions. Furthermore, these materials are also subjected to the microorganism's action and to the effect of the complex factors of the oral cavity. Therefore, the sum of these factors might determine changes in their properties.

Previous studies indicate that the main drawback of these latex based biomaterials is that the toxic products can be ingested and thus to determine various diseases by the accumulation of toxic substances [7]. Latex has an allergenic component that can cause allergic reactions commonly associated with exposure to gloves worn by the orthodontist, causing immediate reactions [8]. There are no studies showing the connections between the cytotoxic and the allergic potential of latex although the substance is being widely regarded as an allergen. There are studies such as that of Lacerda and Santos demonstrating and cytotoxic potential of latex [9-12]. The data correspond to those of our study all four types of biomaterials on the latex demonstrating the cytotoxic potential.

In this study, the negative control group was represented by fragments of dental copper amalgam the dental, that amalgam fillings, were previously shown in the literature to have a strong cytotoxic effect [13]. The cytotoxic potential is due to the presence of copper in the composition. The percentage of viable cells was obtained by comparing the maximum cell density from the negative control group with one of the wells of the study groups. The periods at which the readings were determined short compared with the duration of use the orthodontic devices.

The results of our study on elastic chain are more alarming than those from the literature [11, 14, 15]. The high degree of cytotoxicity of this biomaterial may be due to higher latex content and greater contact with the cells' surface. The results concerning the elastomeric ligatures are similar to those in the literature[17,18,19,20].

Further researches are needed to assess the influence of colors used in the development of cytotoxicity. An in vivo study would have a greater complexity and a number of additional variables, like the presence of saliva, of the bacterial plaque, the effect of the oral pH, temperature and blood flow changes, patient specific immunological reactions.

Conclusions

All materials used in our study determined cellular changes. The elastic chain showed the most pronounced cytotoxic

character. Elastomeric ligatures' cytotoxic character was dependent of their chemical composition, fact demonstrated by two types of elastic modules from the same manufacturer with different cytotoxicity. The study has practical significance by reducing the possibility of adverse reactions due to knowledge cytotoxic nature of the used materials.

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