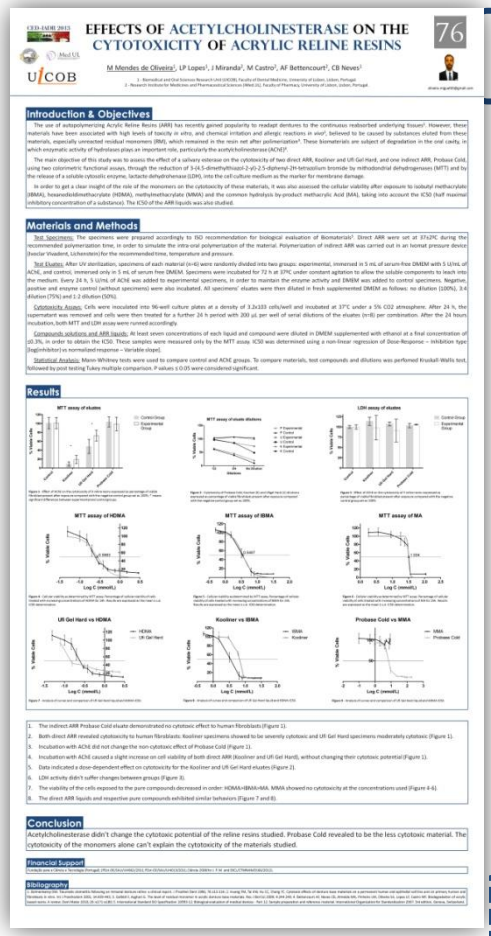
Effects of Salivary Acetylcholinesterase on the Cytotoxicity of Acrylic Reline Resins



CED-IADR 2013 76

EFFECTS OF ACETYLCHOLINESTERASE ON THE CYTOTOXICITY OF ACRYLIC RELINE RESINS

M Mendes de Oliveira[1], LP Lopes[1], J Miranda[1], M Castro[2], AF Bettencourt[2], CB Neves[1]

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Introduction & Objectives

The use of autopolymerizing Acrylic Reline Resins (ARR) has recently gained popularity to readapt dentures to the continuous reabsorbed underlying tissues[1]. However, these materials have been associated with high levels of toxicity in vitro, and chemical irritation and allergic reactions in vivo[2], believed to be caused by substances eluted from these materials, especially unreacted residual monomers (RM), which remained in the resin net after polimerization[3]. These biomaterials are subject of degradation in the oral cavity, in which enzymatic activity of hydrolases plays an important role, particularly the acetylcholinesterase (ACHE)[4].

The main objective of this study was to assess the effect of a salivary esterase on the cytotoxicity of two direct ARR, Kooliner and Ufi Gel Hard, and one indirect ARR, Probase Cold, using two colorimetric functional assays, through the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide by mithodontal dehydrogenases (MTT) and by the release of a soluble cytosolic enzyme, lactacte dehydrothenase (LDH), into the cell culture medium as the marker for membrane damage.

In order to get a clear insight of the role of the monomers on the cytotoxicity of these materials, it was also assessed the cellular viability after exposure to isolatyl methacrylate (IBMA), hexanediolmethacrylate (HDMA), methylmethacrylate (MMA) and the common hydrolysis by-product methacrylic Acid (MA), taking into account the IC50 (half maximal inhibitory concentration of a substance). The IC50 of the ARR liquids was also studied.

Materials and Methods

Test Specimens: The specimens were prepared accordingly to ISO recommendation for biological evaluation of Biomaterials[5]. Direct ARR were set at 37±2ºC during the recommended polymerization time, in order to simulate the intra-oral polymerization of the material. Polymerization of indirect ARR was carried out in an hornat pressure device (Ivoclar Vivadent, Lichtenstein) for the recommended time, temperature and pressure.

Test Eluates: After UV sterilization, specimens of each material (n=6) were randomly divided into two groups: experimental, immersed in 5 mL of serum-free DMEM with 5 U/mL of AChE, and control, immersed only in 5 mL of serum free DMEM. Specimens were incubated for 72 h at 37ºC under constant agitation to allow the soluble components to leach into the medium. Every 24 h, 5 U/mL of AChE was added to experimental specimens, in order to maintain the enzyme activity and DMEM was added to control specimens. Negative, positive and enzyme control (without specimens) were also incubated. All specimens' eluates were then diluted in fresh supplemented DMEM as follows: no dilution (100%), 3:4 dilution (75%) and 1:2 dilution (50%).

Cytotoxicity Assays: Cells were inoculated into 96-well culture plates at a density of 3.2x103 cells/well and incubated at 37ºC under a 5% CO2 atmosphere. After 24 h, the supernatant was removed and cells were then treated for a further 24 h period with 200 µL per well of serial dilutions of the eluates (n=8) per combination. After the 24 hours incubation, both MTT and LDH assay were runned accordingly.

Compounds solutions and ARR liquids: At least seven concentrations of each liquid and compound were diluted in DMEM supplemented with ethanol at a final concentration of ≤0.3%, in order to obtain the IC50. These samples were measured only by the MTT assay. IC50 was determined using a non-linear regression of Dose-Response – Inhibition type (log(inhibitor) vs normalized response – Variable slope).

Statistical Analysis: Mann-Whitney tests were used to compare control and AChE groups. To compare materials, test compounds and dilutions was performed Kruskal-Wallis test, followed by post testing Tukey multiple comparison. P values ≤ 0.05 were considered significant.

Results

1. The indirect ARR Probase Cold eluate demonstrated no cytotoxic effect to human fibroblasts (Figure 1).
2. Both direct ARR revealed cytotoxicity to human fibroblasts: Kooliner specimens showed to be severely cytotoxic and Ufi Gel Hard specimens moderately cytotoxic (Figure 1).
3. Incubation with AChE did not change the non-cytotoxic effect of Probase Cold (Figure 1).
4. Incubation with AChE caused a slight increase on cell viability of both direct ARR (Kooliner and Ufi Gel Hard), without changing their cytotoxic potential (Figure 1).
5. Data indicated a dose-dependent effect on cytotoxicity for the Kooliner and Ufi Gel Hard eluates (Figure 2).
6. LDH activity didn't suffer changes between groups (Figure 3).
7. The viability of the cells exposed to the pure compounds decreased in order: HDMA>IBMA>MA. MMA showed no cytotoxicity at the concentrations used (Figure 4-6).
8. The direct ARR liquids and respective pure compounds exhibited similar behaviors (Figure 7 and 8).

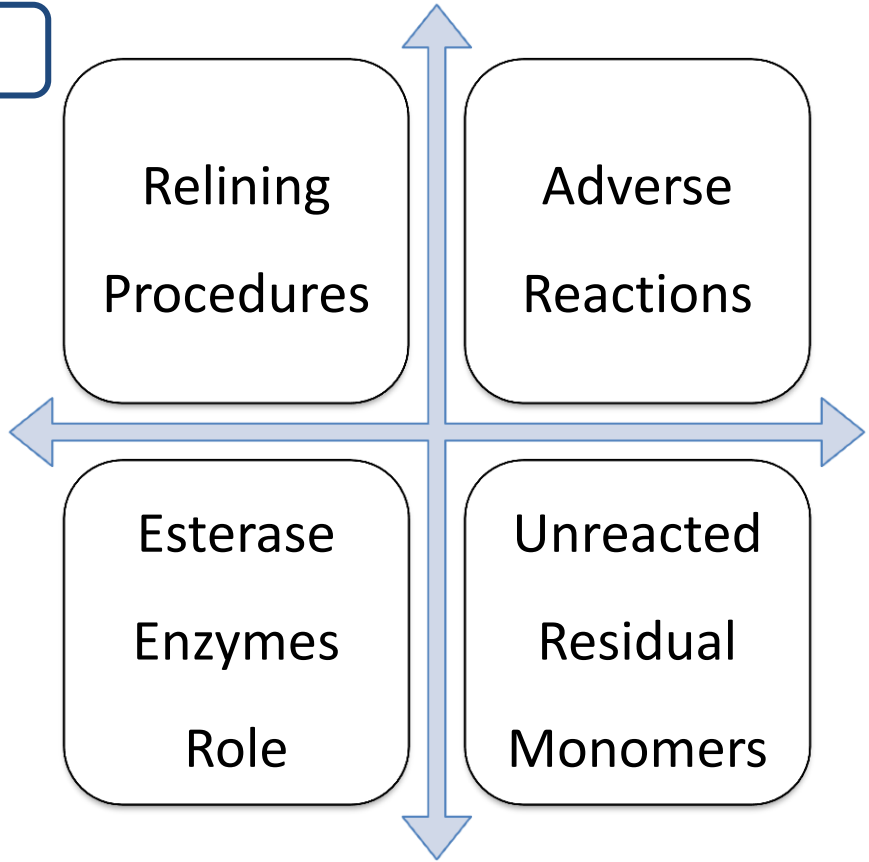
Conclusion

Acetylcholinesterase didn't change the cytotoxic potential of the reline resins studied. Probase Cold revealed to be the less cytotoxic material. The cytotoxicity of the monomers alone can't explain the cytotoxicity of the materials studied.

Financial Support

Bibliography

State of the Art



Bohnenkamp DM. Traumatic stomatitis following an intraoral denture reline: a clinical report. J Prosthet Dent 1996; 76:113-114; Huang FM, Tai KW, Hu CC, Chang YC. Cytotoxic effects of denture base materials on a permanent human oral epithelial cell line and on primary human oral fibroblasts in vitro. Int J Prosthodont 2001; 14:439-443; Bettencourt AF, Neves CB, Almeida MA, Pinheiro LM, Oliveira SA, Lopes LP, Castro MF. Biodegradation of acrylic based resins: A review. Dent Mater 2010; 26: e171-e180

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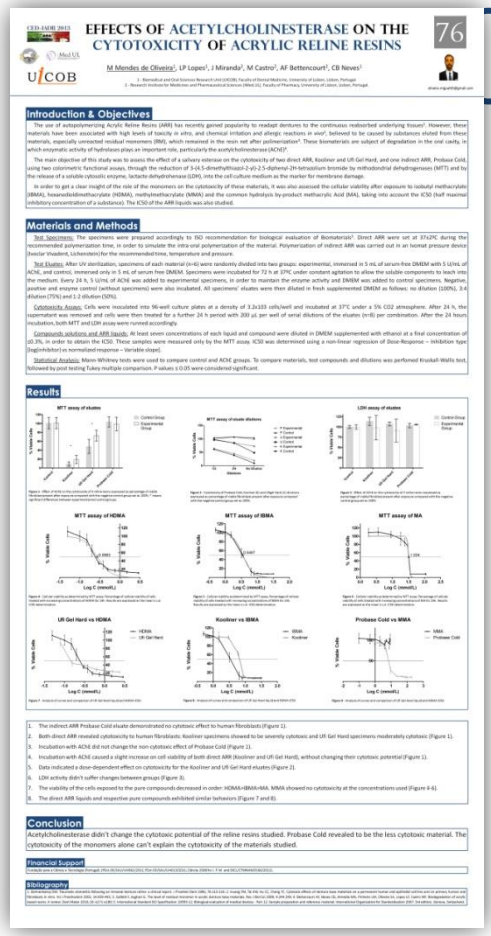
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Introduction & Objectives

Materials and Methods

Results

Conclusion

Financial Support

Bibliography

Materials & Methods

Kooliner (K)

GC America Inc

Ufi Gel Hard (U)

VocoGmbH

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Probase Cold (P)

Ivoclar Vivadent AG

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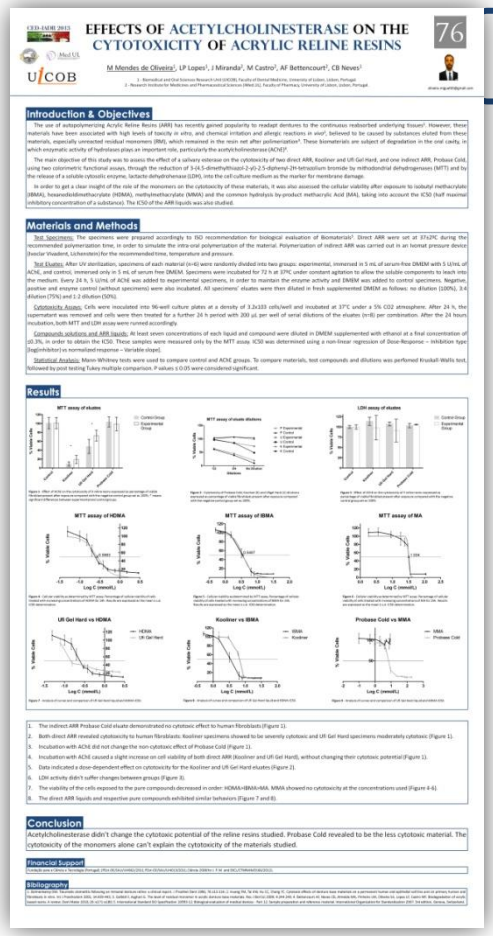

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Conclusion

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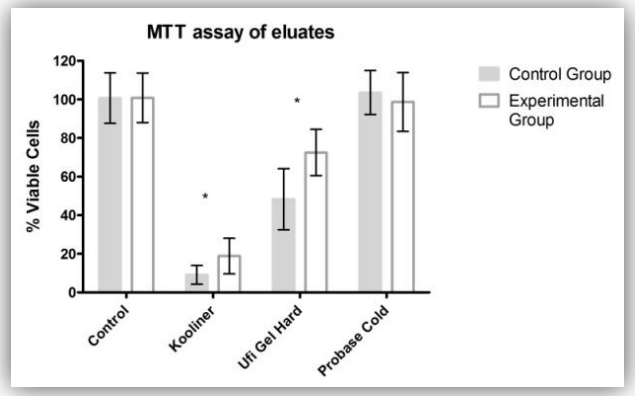
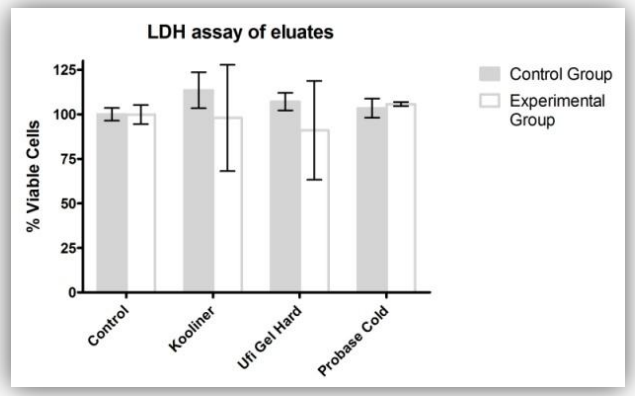
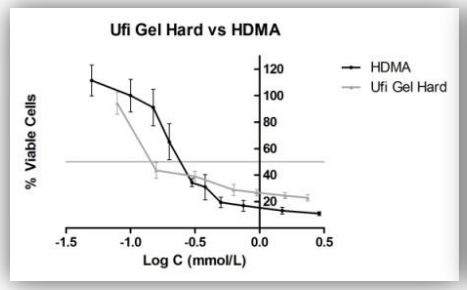
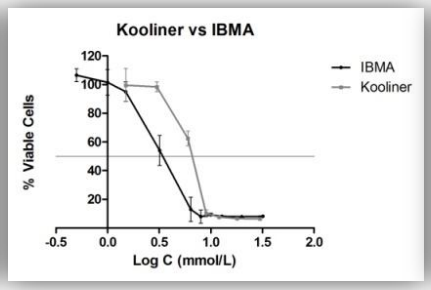
Bibliography

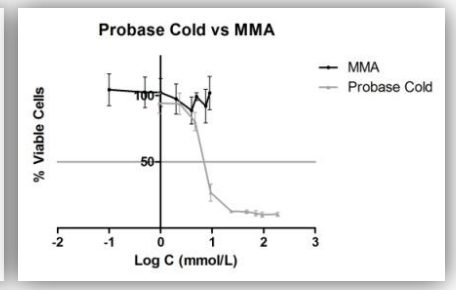
Results











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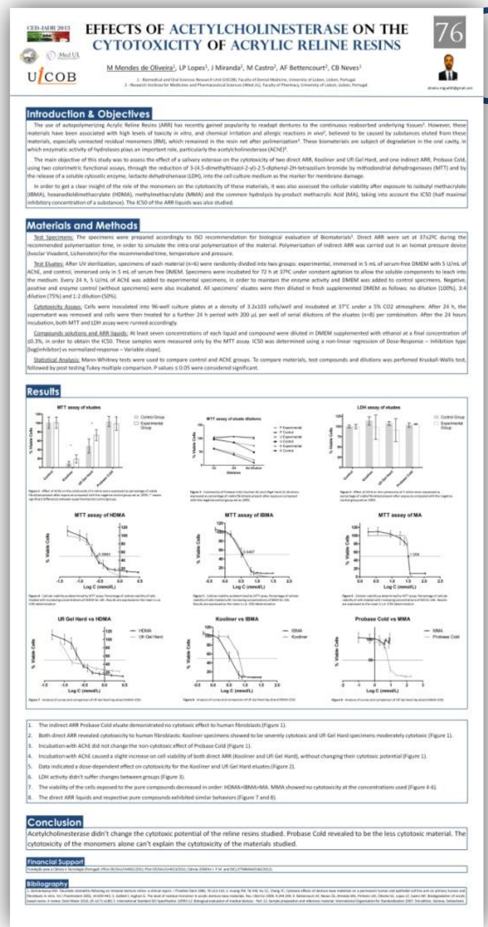

Conclusions

Acetylcholinesterase didn’t change the cytotoxic potential of the reline resins studied.

Probase Cold was the less cytotoxic material.

HDMA > IBMA > MA

Financial Support: Fundação para a Ciência e Tecnologia (Portugal): (PEst-OE/SAU/UI4062/2011; PEst-OE/SAU/UI4013/2011; Ciência 2008 for J. P. M. and EXCL/CTMNAN/0166/2012).