REPORT ON *IN VITRO* DETERMINATION OF BIOCOMPATIBILITY OF COMMERCIAL DENTAL MATERIALS

For: **GLASS CARBOMER PRODUCTS B.V.**
P.O. Box 539
2300 AM Leiden
The Netherlands

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From: DR KATHRYN HURRELL-GILLINGHAM
BIOMATERIALS RESEARCH GROUP
SCHOOL OF CLINCIAL DENTISTRY
UNIVERSITY OF SHEFFIELD
SHEFFIELD S10 2TA

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Reviewed by: **DR PAUL HATTON**
CENTRE FOR BIOMATERIALS & TISSUE ENGINEERING,
SCHOOL OF CLINICAL DENTISTRY
UNIVERSITY OF SHEFFIELD,
SHEFFIELD S10 2TA

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1. Introduction

Dental materials are generally considered to be safe, although questions regarding the biocompatibility of specific materials have been raised for several decades. The focus was initially on the safety of amalgam due to the toxic mercury component, and it is likely that this issue has contributed to the increased popularity of resin-based composite restorative materials. Resin composites and related hybrid materials (e.g. so-called compomers) are not without their own biocompatibility issues because of fears regarding monomer release and adverse reaction. This has led some to question the rise in popularity of resin-based materials and instead look to developments in traditional glass-ionomer (GIC) technology to deliver safe and effective restorative materials for dentistry. Advances here have led to the development of glass carbomers that may be rapidly matured to form a durable and aesthetic restoration with significantly improved properties and greater longevity than conventional GICs. It is also predicted that, if prepared in accordance with manufacturers' instructions, glass carbomers will exhibit improved biocompatibility compared to existing restorative materials including conventional GICs (that are already considered among the most biocompatible materials). No detailed comparative studies of the in vitro biocompatibility of glass carbomers have been reported to date. The aim of this short project was to evaluate the *in vitro* biocompatibility of a range of restorative dental materials including glass carbomer using cell culture methods related to the in vitro tests described in ISO10993-5 and ISO7405.

2. Materials and methods

2.1 Materials

Discs (10 mm diameter by 1 mm depth) of materials (see table 2.1) were produced and an adhesive, gloss or glaze applied to one surface using the manufacturers instructions. The resulting discs were then sterilised by autoclaving (15 min at 121° C/15 psi) prior to direct biocompatibility assessment.

Table 2.1: Materials employed for the study

Material	Type	Cure	Manufacturer
Equia GC Fuji IX GP extra G-Coatplus	 Glass ionomer Adhesive	 Self Light (20 s)	 GC Europe N.V B-3001 Leuven, Belgium
Z100 Adper Scotchbond 1XT	Composite Adhesive	Light (40 s) Light (10 s)	3M ESPE AG Seefeld, Germany.
Glass Carbomer ®	Nano-filled carbomised glass ionomer	Light (90 s)	First Scientific Dental GmbH Elmshorn, Germany.
Ketac Molar Aplicap Ketac Glaze	Glass-ionomer Glaze	Self Light (10 s)	3M ESPE AG Seefeld, Germany.
Vitremer core buildup Vitremer Finishing Gloss	Tri-cure glass ionomer Gloss	Light (40 s) and Self Light (20 s)	3M ESPE AG Seefeld, Germany.

All materials were allowed to cure for 1 h before sterilisation.

2.2 Tissue culture

Biocompatibility was investigated using mouse fibroblast (L929) seeded into wells of a 24 well plate containing test samples. This cell line was selected for its reproducibility, and its history of successful use in the evaluation of the biocompatibility. Standard published methods were applied (seeding density of 1.25 x 10^4 cells.ml^{-1} in a total volume of 2.0 ml medium). A non-material control (tissue culture plastic) was included in the experimental series for comparison. The materials and cells were incubated at 37°C in a 5% CO_2 atmosphere for 72 h. Alamar Blue™

assay was carried out to determine the respiratory activity of cultures maintained in the presence of the different materials (all experiments were n=4,).

3. Results

Quantitative analysis (Figure 1) of the cell viability was assessed using an Alamar™ blue assay. L929 cells did not appear to remain viable in the presence of some test materials. In addition, the application of the appropriate proprietary adhesive, gloss or glaze resulted in a further decrease in cell viability in all materials accept for the Equia system. The greatest cellular activity was detected in the presence of the Glass Carbomer and the Ketac Molar samples. However, the application of a gloss to the surface of the Ketac Molar cement resulted in a decrease in cell respiratory activity. Contact testing with vitremer resulted in the lowest cellular response and the presence of a gloss had little or no impact of the cells.

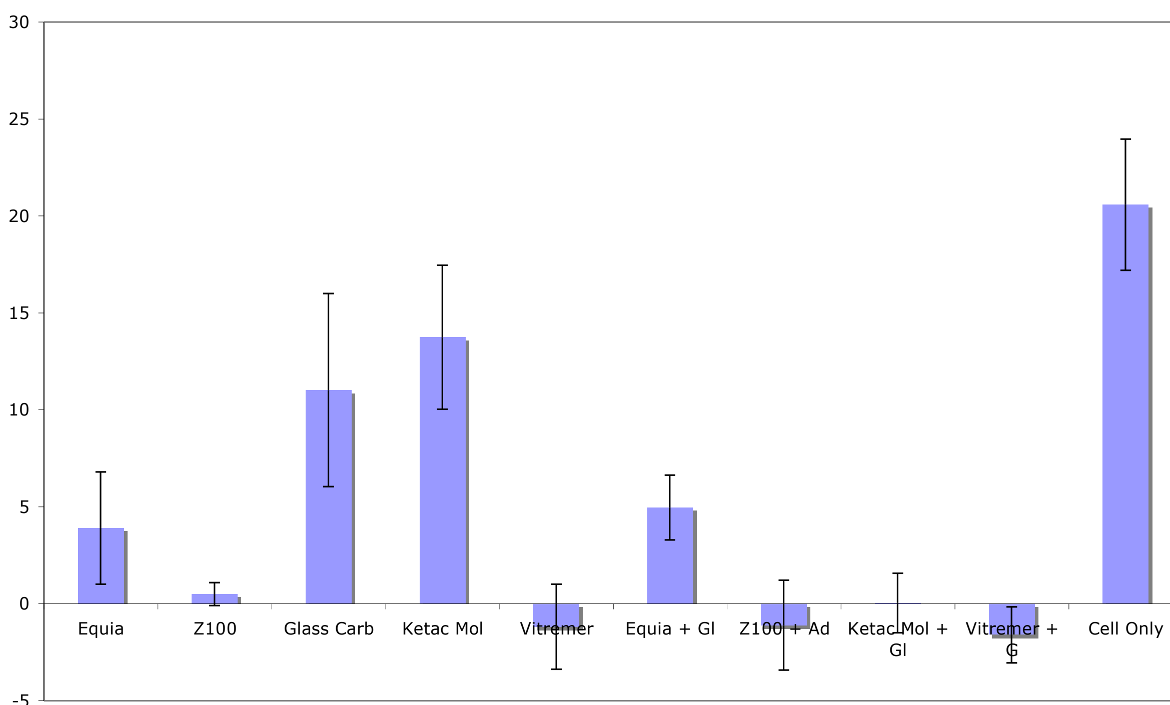

Figure 1: Percentage Alamar Blue reduction of L929 cells in direct contact with various dental restorative materials.

Ranked as a mean percentage of the control wells (tissue culture plastic), the order from most to least biocompatible under these *in vitro* conditions was: Ketac Molar (67%) > Glass Carbomer (54%) > Equia + glaze (24%) > Equia (19%) > Z100 (2%) > Ketac Molar + Glaze (0%) > Z100 + Adhesive (-5%) > Vitremer (-6%) > Vitremer + Gloss (-8%). The raw data can be found in appendix 1.

4. Discussion & conclusions

The quantitative analysis showed that the best cellular was observed in the Ketac Molar and Glass Carbomer samples. Vitremer had the lowest respiratory activity. The application of the appropriate proprietary adhesive, glaze or gloss generally resulted in a decrease in cellular respiration.

Appendix 1

Table 1: Respiratory activity of L929 cells in direct contact with dental materials.

	Mean Respiratory Activity	**Standard Deviation**
Equia	3.90	2.89
Z100	0.50	0.60
Glass Carb	11.02	4.98
Ketac Mol	13.73	3.72
Vitremer	-1.18	2.20
Equia + GI	4.96	1.67
Z100 + Ad	-1.09	2.32
Ketac Mol + GI	0.04	1.53
Vitremer + G	-1.60	1.45
Control (Tissue Culture Plastic)	20.58	3.39