CYTOTOXIC EFFECTS OF RESTORATIVE BIOMATERIALS ON DENTAL PULP – A SYSTEMATIZED REVIEW

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ABSTRACT
Background: The most important cause of pulpal damage due to restorative procedures/materials is the toxic substances release from the material. Understanding the cytotoxicity and various tests to determine these effects helps in validating and better decision making in day to day dental practice. Objectives: To assess the cytocompatibility of commonly used restorative materials and methods to detect the cytotoxic effects on dental pulp. Methods: An electronic database search [MEDLINE, EMBASE, PUBMED] complimented by journal database [Elsevier, science direct, SAGE] and manual search was conducted to identify the reviews, systematic reviews and meta-analysis reporting cytotoxic tests or cytotoxic effects of restorative materials pertinent to dental pulp. No language restrictions or date were used initially. Results: The initial search provided 1572 titles, complimented with 285 titles from manual search and journal database. Following the final filtering process 69 studies were finally selected and included in this review. No systematic reviews or meta-analysis were found. Conclusion: Among the various cytotoxicity tests, dentin barrier test and tooth slice culture methods are promising in detecting the cytotoxic effects of the dental materials on pulp. Most of the currently used dental materials are not cyto compatible to pulp if placed directly on pulp or the remaining dentin thickness is less than 0.5mm.

KEYWORDS: Biocompatibility, Dental materials, Invitro test, Cytocompatibility, Cytotoxicity assay, Preclinical tests.
INTRODUCTION

Restorative dental materials are the foundation for replacing diseased tooth or lost tooth structure. The primary objective of using restorative material is to restore form and function. All four major classes of biomaterials: Metals, Polymers, Ceramics, Composites are used for restoration. Various classification systems are used to categorize the restorative dental materials [based on use, fabrication, longevity]. The most commonly used classification of restorative dental material is as follows.

Table 1: Classification on Restorative materials

<table>
<thead>
<tr>
<th>Direct restorative materials</th>
<th>Indirect restorative materials</th>
<th>Auxiliary dental materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonding &amp; Bonding agents</td>
<td>Casting alloys</td>
<td>Impression materials, Gypsum products, Dental waxes</td>
</tr>
<tr>
<td>Resin composites</td>
<td>Dental ceramics</td>
<td>Finishing, Polishing materials</td>
</tr>
<tr>
<td>Dental cements</td>
<td>Prosthodontic polymer resins</td>
<td>Cutting, grinding, abrasive materials</td>
</tr>
<tr>
<td>Dental Amalgam</td>
<td>Wrought metals</td>
<td>Preventive materials</td>
</tr>
</tbody>
</table>

The selection of material for clinical use is based on mechanical, physical, biological properties of the material intent to use. Three major factors are considered as foundation for the success of restorative dental material, they are 1. Material properties, 2. Design, 3. Biocompatibility.

Williams (2008) defined Biocompatibility as “the ability of a biomaterial to perform its desired function with respect to a therapy, without eliciting and undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation and optimizing the clinically relevant performance of that therapy”. Biocompatibility (lack of harmful effects) is a measure of body’s biological response to a material used in specific application. It is a property of a material interacting with its environment. The biological response to a material will change if change occurs in the host or the application of the material.

Biocompatibility testing is an important part of obtaining regulatory body approval to market any medical device or materials intent to use in human body. Three basic categories of testing is done to ensure that the material is biocompatible, they are Cytotoxicity test, sensitization,
irritation & intracutaneous reactivity. Among this three cytotoxicity is the most sensitive of biocompatibility test. It is said that if a sample fails only one of Biocompatibility test 90% of time cytotoxicity test fails. Cytotoxic effects is the quality of the material being toxic to cells. It is the degree to which the material possess a specific destructive action on certain cells. Cytotoxic effects of restorative dental materials are evaluated by series of test like comparative analysis, surface degradation tests, cell culture tests, clinical testing in humans and animal models. Several factors determine the cytotoxic effects of restorative materials. This includes chemical nature of its components, physical nature of the components, type and location of tooth that exposed to restorative material, duration of exposure, surface characteristics, amount and nature of substances eluted from the material, oral & host environment, and depth of the restoration. It is hypothesized that restorative materials may directly affect plural tissue or play an auxiliary role in causing sub lethal changes to pulpal tissues. The exact and complete biological effects of restorative material on dental pulp are still not clear. Thus the objective of this report is to systematically review the literature that described Cytocompatibility, of commonly used restorative biomaterials on dental pulp and various cytotoxicity test to detect the cytotoxic effects.

MATERIALS AND METHODS

Review Protocol: The PICO question used for this systematic reviews was to investigate the various cytotoxic effects, cytocompatibility, biocompatibility of restorative materials and methods to test cytotoxicity. The focused review question used in this review was shown in the table-1.

Search strategy: A comprehensive literature search published up to March 6th 2015 was performed on the article databases: Ovid Medline [from 1946], EMBASE [from 1947] PubMed. The search strategy used a combination of medical subject headings (MeSH) terms and keywords for Medline, PubMed and EMBASE. The keywords and MeSH terms used for the search were shown in the table-1.Further the main search was complimented by searching in additional journal databases[ Elsevier, science direct, SAGE] to identify additional literatures. In addition, a hand search strategy was performed by the authors from the citation/reference list of the primary studies and reviews. No restrictions were applied in the search initially. Later filters [reviews, systematic reviews, Meta-analysis] were used.
Inclusion & Exclusion criteria: Systematic reviews, meta-analysis, reviews were included in this review provided that the study presented or reported cytotoxic effects/cytotoxicity tests/biocompatibility/biocompatibility test of restorative materials on dental pulp and the study was reported in English and peer reviewed literature. Original research reports, non-English literature, studies reported on endodontic materials/dental implants/bleaching/whitening are excluded from this review. The comprehensive list of included and excluded criteria were shown in table-2.

Table-2. Search Strategy

<table>
<thead>
<tr>
<th>Focus Question: What are the various cytotoxic effects exhibited by the different restorative materials on vital Pulp during restorative treatment procedures?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population: Vital Pulp #1 (vital tooth) OR ((tooth) AND vital)) OR dental pulp cavity) OR odontoblasts) OR dental pulp) OR dental pulp cells) OR (dental mesenchymal cells) OR ((dental) AND pulp)) OR ((dental pulp) AND cells) OR ( dental caries)</td>
</tr>
<tr>
<td>Intervention/ Exposure: Restorative treatment procedure #2 (dental atraumatic restorative treatment) OR (denture,partial, fixed) OR (tooth preparation) OR (cavity preparation) OR (dental cavity preparation) OR(inlays) OR (onlays) OR (crowns) OR ( bridges) OR (dental bonding) OR (dental pulp exposure) OR (dental pulp capping)</td>
</tr>
<tr>
<td>Comparison: Different restorative materials #3 (dental restorative material) OR provisional restorative material) OR intermediate restorative material) OR permanent restorative material) OR final restorative material) OR dental restoration, permanent) OR dental restoration, temporary) OR dental restorative materials) OR indirect restorative materials) OR dental restoration) OR restorative biomaterials) OR dental materials) OR biocompatible materials) OR restorative materials) OR dental application materials) OR biocompatible dental materials) OR resin cements) OR dental cements) OR resin composites) OR (cementation) OR glass ionomer cements) OR composite resin) OR dentin-bonding agents) OR methacrylates) OR dental amalgam) OR (pulp capping and pulpotomy agents) OR dental atraumatic restorative treatment) OR crowns) OR dental porcelain) OR ceramics) OR dental bonding) OR nanoparticles) OR dental alloys) OR metal ceramic alloys) OR chromium alloys) OR gold alloys) OR dental clasp) OR polymers) OR polyacrylic acid cement) OR zinc oxide eugenol cement) OR composites) OR cementum) OR fluorides) OR calcium hydroxide) OR zinc phosphate cement) OR dental prosthesis retention) OR denture, partial, fixed) OR ((dental) AND (((varnish) OR base) OR sub base))) OR dental pins)</td>
</tr>
<tr>
<td>Outcome: Cytotoxic effects #4 (cytotoxic) OR ((cytotoxic AND effects)) OR biocompatibility) OR toxicity) OR safety) OR biocompatible) OR cytology) OR dental leakage) OR materials testing) OR apoptosis) OR cell survival) OR pulp death) OR cytotoxicity, immunologic) OR cytotoxicity tests, immunologic) OR safety) OR biocompatibility test) OR safety test) OR chemical safety) OR hazard analysis) OR pulp necrosis)</td>
</tr>
<tr>
<td>Search Combination</td>
</tr>
<tr>
<td>Database search</td>
</tr>
<tr>
<td>Language</td>
</tr>
<tr>
<td>Inclusion Criteria</td>
</tr>
</tbody>
</table>
Screening methods and data extraction: Data were extracted based on authors, year of publication, characteristics (type of restorative materials, type of tests, type of preclinical models, main observation of the study, limitations of the study).

Study Quality Assessment: Selected systematic reviews, and meta-analysis were subjected to AMSTAR [Assessing the methodological quality of systematic reviews] tools to qualitatively score the reviews.

RESULTS

Table-3: Flow chart of search Methodology

The titles and abstracts of 1857 articles [initial search] were screened to assess their suitability for full text review. After removing the duplicates [n= 421], 1168 articles were excluded based on the title review. Thus 268 articles were eligible for the abstract screening. Of those 115 articles were excluded based on the exclusion criteria. Among the 153 full text articles reviewed, 84 were excluded [The details of the excluded articles and the reason for exclusion were shown in supplemental table-2] leaving 69 full text articles to be included in this review. The flow chart describing the screening process based on PRISMA guidelines was shown in table -3. Due to the paucity of the systematic reviews and meta-analysis [0 studies] quality assessment was not undertaken. Because most of the selected studies are narrative reviews no characteristic table is used in this literature review. Observations from
the selected full text articles are synthesized and presented in discussion as follows:
cytotoxicity test, screening methods, material-cell interface, cell lines, endpoints and current
status.

**DISCUSSION**

Biocompatibility testing of any materials involves four sequential phases of evaluation. Phase
1 and 2 [cytotoxicity tests] are considered as foundation test. The material which successfully
pass through this tests are considered for phase 3 and phase 4. Restorative dental materials
are constantly evolving throughout the years with dispelling several myths like acid etching
of vital dentin will devitalize underlying pulp to calcium hydroxide is the only pulp
protecting material. The pulpal response to any dental materials depends on various factors
like size and concentration of molecules, density, length, diameter of the dentinal tubules,
effect of temperature, remaining dentin thickness and longevity of the restoration. The life
span of commonly used restorative materials are shown in the table 4.

<table>
<thead>
<tr>
<th>Table: 4 Longevity of the commonly used restorative materials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dental Amalgam</strong> – approximately <strong>14 years</strong></td>
</tr>
<tr>
<td><strong>Cast gold</strong> – <strong>20 years</strong></td>
</tr>
<tr>
<td><strong>Ceramic crowns</strong> - <strong>15 years</strong></td>
</tr>
<tr>
<td><strong>Ceramic inlays</strong> - <strong>10 years</strong></td>
</tr>
<tr>
<td><strong>Gold foil, mat gold</strong> – <strong>24 years</strong></td>
</tr>
<tr>
<td><strong>Compomer</strong> - <strong>10 years</strong></td>
</tr>
<tr>
<td><strong>PFM crowns</strong> - <strong>20 years</strong></td>
</tr>
<tr>
<td><strong>GIC</strong> - <strong>8 years</strong></td>
</tr>
<tr>
<td><strong>Resin composites</strong> – <strong>class1, 2: 10 years, class 3, 4,5: 15 years</strong></td>
</tr>
</tbody>
</table>

On an average restorative materials are expected to be in service for at least ten years. Any
restorative materials that are placed adjacent to vital pulp can induce biological effects. These
effects are controlled by the components that are released from the material and the pulpal
response to those components. The fig-1 shows three possible ways a cytotoxins/ substance
release from restoration follows. The three possible pathways a restoration can Thus the
cytotoxicity effects of these materials on dental pulp is more vital in choosing an appropriate
restorative material for a given clinical situation. The details of various methods to determine
cytotoxicity/cyto compatibility, factors in choosing appropriate test and current status of the
commonly used restorative materials are discussed in the following paragraphs.
Fig:1 Possible pathways a leached/ released components from a restoration can happen. [black arrow- to pulp, blue arrow – to oral cavity, green arrow- to periodontium]

1. Cytotoxicity tests

The term cytotoxicity is used to describe the cascade of molecular events that cause functional and structural damage to cells. Throughout the years various assays and protocols were developed to test the cytotoxic effects of biomaterials. The rationale behind doing a cytotoxicity test is to determine how a material sample affects a particular cell type. The primary criteria of these tests are the material must not affect the cell number, cell growth, genetic integrity, membrane integrity, genetic expression and enzymatic activity of the cells. The cytotoxicity tests are broadly categorized into viability assays, survival assays, metabolic assays, transformation assays, and inflammation assays. Different assays currently in use are showed in the table-5.

Table: 5 Various currently use assays that measure cytotoxic effects

<table>
<thead>
<tr>
<th></th>
<th>Assays based on membrane integrity.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Dye exclusion assays.</td>
</tr>
<tr>
<td>3</td>
<td>Dye uptake assays.</td>
</tr>
<tr>
<td>4</td>
<td>Chromium uptake assays.</td>
</tr>
<tr>
<td>5</td>
<td>Enzyme release assays.</td>
</tr>
<tr>
<td>6</td>
<td>Assays based on cellular respiration.</td>
</tr>
<tr>
<td>7</td>
<td>Assays based on radioisotope incorporation.</td>
</tr>
<tr>
<td>8</td>
<td>Assays using labelled nucleotides.</td>
</tr>
<tr>
<td>9</td>
<td>Assays using labelled phosphatases.</td>
</tr>
<tr>
<td>10</td>
<td>Assays based on calorimetric test.</td>
</tr>
<tr>
<td>11</td>
<td>Assays based on luminescence test.</td>
</tr>
<tr>
<td>12</td>
<td>Assays based on apoptosis</td>
</tr>
</tbody>
</table>
Among the various cytotoxicity assays the most commonly used one is the tests that based on membrane integrity. The membrane integrity can be evaluated by the uptake of dyes. Dye such as naphthalene blue, trypan blue, erythrosine, neutral red, diacetyl fluorescin were commonly used in such assays.

2. Screening methods

ISO 10993-5: 2009 presents the general guidelines for cytotoxic screening. The ISO 7405:2008 details the screening protocol pertinent to restorative dental materials. Based on the regulatory body guidelines, four screening methods are recommended. They are 1. Direct cell culture and culture extract testing [barrier screening assays], 2.Agar diffusion testing [Tissue culture overlay test], 3.Filter diffusion testing [Millipore cellulose acetate method], 4. Dentin barrier testing [Model cavity method].

About twenty different cell culture techniques were used to assess the cytotoxic effects of biomaterials using the barrier screening assays. The main strategy behind this screening method is to test the cytotoxicity of individual components of restorative material by placing them directly onto cells in a monolayer culture for short duration usually more than 24 hours. Using the dose response curve, the cytotoxic potential of each ingredient in the material is calculated.

However the disadvantages in using this method are time consuming, technique sensitive, troublesome in interpretation, subjective judgement so often not clinically relevant. The direct use of cell and colony counting are the least reliable method to evaluate the cytotoxic effects.

Agar diffusion testing is the most established testing methods since it is used for more than 50 years. The method uses permanent cell lines and test the ability of leachable components of restorative material to diffuse through dentinal tubules. This is assessed by staining the permanent cell lines with neutral red vital stain dye covered with a layer of agar and on which the test material is incubated for 24 hours. Fig-2 shows the agar overlay test illustration. The presence of leachable toxic substance is confirmed by the loss of dye within the cells. Though the screening method costs less, the accuracy of the test depends on the material components to diffuse through agar monolayer thus jeopardizing the outcome of test results.
In the Millipore cellulose acetate method 0.45μm filters are used. On one side of the filter cells are grown and on the other side test materials were placed. Based on the appearance of filters at the material and cell interface, scoring were done based on ISO 7405 guidelines to classify the cytotoxic response. The test method is based on enzyme activity. Glucose 6 phosphatase dehydrogenase, cytochrome oxidase, lactate dehydrogenase are used as assay endpoints.

Dentin barrier test or model cavity method was based on the idea by Outwaiitte et al [1974]. This method mimics the invivo oral environment and test the ability of the material to diffuse through dentinal tubules. Thus ideal for testing cytotoxic effects of the material on dental pulp. The first dentinal barrier test was devised by Tyass [1977]. The author simulated dental cavity consist of Pyrex cylinder projecting through the lid of 30 mm petri dish. This method later [1989] become British standard for testing restorative materials. The DBT [Dentin barrier test] is the ideal test for evaluating the restorative material components that are responsible for pulpal effects. This is highly recommended method to test the cytocompatibility of any new restorative material. The schematic illustration of dentin barrier test is showed in the figure3.
3. Material-Cell Interface in Cytotoxicity Test

There are three major possible ways to design the material-cell contact in invitro test. 1. Direct, 2. Indirect or through an eluate. In direct method cells can be placed onto material/around the material/ materials onto cells. In the indirect method a barrier is used to separate cells and test material. The schematic illustration of the direct and indirect design is shown in the figure-4.

The material-cell interface plays a major role in invitro cytotoxicity test outcomes and its clinical importance. The classical example is Zinc oxide eugenol [ZOE] based cements. Early invitro tests had reported that they are severe toxic to pulp. However it was disproved by the successful clinical use in restoring cavities. The invivo pulpal studies reported low toxicity. The non-direct placing of ZOE by using dentin barrier in invitro test demonstrated the apparent low toxicity of this material. The design plays a vital role in implication of the
invitro test. The following table-6 shows the recommended cell-material interface based on the nature of test material.

**Table: 6 Recommended cell-biomaterial interface**

<table>
<thead>
<tr>
<th>Biomaterial Form</th>
<th>Cell-material interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid</td>
<td>Direct/indirect</td>
</tr>
<tr>
<td>Liquid, powder, fibers</td>
<td>Indirect</td>
</tr>
</tbody>
</table>

Based on the material-cell interface, the cytotoxicity tests can be categorized as direct tests and indirect tests. The following table-7 shows the different tests used in each categories.

**Table: 7 Cytotoxicity tests based on material-cell interface**

<table>
<thead>
<tr>
<th>Direct</th>
<th>Indirect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTT assay</td>
<td>Agar overlay test</td>
</tr>
<tr>
<td>NBT assay</td>
<td>Millipore filter test</td>
</tr>
<tr>
<td>XTT assay</td>
<td>Dentin barrier test</td>
</tr>
<tr>
<td>WST assay</td>
<td></td>
</tr>
<tr>
<td>Alamar blue assay</td>
<td></td>
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</tbody>
</table>

4. **Cell Lines used in cytotoxicity test:** Two types of cell lines can be used to determine the cytotoxic effects of the restorative biomaterials: primary cell lines [grow for only limited time in culture] and permanent cell lines [grow more or less indefinitely in culture]. The choice of cell line is a controversial and so far no clear recommendations are guidelines are available. ISO 10993:1999 preferred the use of established cell lines like mouse fibroblasts [clone L929], Balb/3T3, WI-38 for cytotoxicity tests.

Various commonly used cell lines are shown in following table-8

**Table: 8 Commonly used cell lines in cytotoxicity tests**

<table>
<thead>
<tr>
<th>Source</th>
<th>Continuous cell lines</th>
<th>Source</th>
<th>Primary cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>L929, P1534,3T3</td>
<td>Mouse</td>
<td>Synovial fibroblasts, thymocytes</td>
</tr>
<tr>
<td>Rat</td>
<td>Walker 245</td>
<td>Rat</td>
<td>Synovial fibroblasts</td>
</tr>
<tr>
<td>Monkey</td>
<td>VERO</td>
<td>Chick</td>
<td>Embryo, embryonic heart fibroblasts, calvarial osteoblasts</td>
</tr>
<tr>
<td>Human</td>
<td>HeLa,KB,WI-38,IMR-90, MRC-5</td>
<td>Rabbit</td>
<td>Corneal cells, peripheral blood monocytes, peritoneal and alveolar macrophages</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>Synovial fibroblasts, peripheral blood lymphocytes and granulocytes, endothelial cells, corneal cells, osteoblasts</td>
</tr>
</tbody>
</table>
However these cell lines don’t represent oral tissues. Ideally odontoblast cells should be used for cytotoxicity screening of restorative materials, but it is very difficult in growing this cells separated from dentin matrix. Various researches had attempted to transform primary oral explanted cells in culture into permanent cell lines but their phenotypic resemblance are questionable. The pulp fibroblasts are readily cultured, the drawback is their greater variability from culture to culture in the means of growth characteristics and sensitivity to cytotoxins. To overcome this existing drawbacks tooth slice culture method was introduced. It has the benefit of maintaining identical experimental conditions similar to oral environment. Further it allows to evaluate the restoration procedures and restoration variables. So, far these variables were not able to be measured in animals and clinical trials. Murray (2000) further suggested that tooth slice culture eliminates bacterial contamination the factor that often exaggerate the toxic effects of test materials. However tooth slice culture method requires further development and optimization.

5. Endpoints of cytotoxicity assays
Assay endpoints are categorized into metabolic impairment assay and membrane integrity assay. Various endpoints measured are as follows: assessment of cell damage by morphological means, measurement of cell damage, measurement of cell growth, measurement of specific aspects of cell metabolism.

6. Current cytotoxic status of commonly used restorative materials
A. Amalgam: Amalgam is been used for restoration for more than 150 years despite various controversies and speculations over these years. Dental amalgam are considered as either inert or mildly irritating to dental pulp. These was demonstrated by manly 1942, schroff 1946, 1947, schour 1955, siberkweit 1955, masler 1956, welder 1956 by conducting experiments in dogs, rats and humans.

Later cell culture screening tests demonstrated that free mercury in amalgam and copper is toxic to cells. Various experiments concluded that mercury from amalgam in humans and dogs do not reach pulp. Stanley in 1991 confirmed with his experiment that any pulpal response to amalgam is due to the insertion procedure [pressure of condensation] rather than the material itself. This was further confirmed by the usage tests, that the pulpal response to amalgam in shallow, deep cavities that are appropriately lined shows minimal response and amalgams rarely cause irreversible damage to the pulp. The current recommendation is
cavities with 0.5mm to 1 mm remaining dentine on cavity floor should be appropriately lined before placing amalgam restoration.

B. Resin Based composites.
Resin based composites are combination of organic and inorganic phase. Invitro cytotoxicity tests had demonstrated moderate cytotoxic effects. Composite materials has shown chronic inflammatory response in invivo and cytotoxic in cell culture. Past two decades the pulp and dentin reactions to composites are attributed to bacterial leakage rather than cytotoxic effects. Pulp studies of individual components by Stanley et al showed slight but varied response. Though early researches showed severe pulp reaction to composites, most studies after 1985’s shown no or moderate reaction. Almost all of the major components of composites are cytotoxic in invitro tests. But they are tested as bulk monomer. Histologic studies confirmed that even low concentration of residual monomer molecules show moderate degree of cytotoxicity on pulpal tissues. Goldberg (2008) confirmed that low to moderate pulpal inflammatory response after 3 days to chemically and light cure resin composite in cavities with approximately 0.5mm remaining dentin thickness. However the reaction was not seen after 5-8 weeks. Further it is accompanied by reparative dentin formation. Invivo studies in recent past had suggested that complete or incomplete cured resins caused little irritation to pulp if an adequate marginal seal is present. The cytotoxicity of the cured composite is based on the extent of release of these components from the composites and dentin barrier effectively reduces the ability of the released components to reach the pulp. Current recommendation is to place a protective liner or bonding agent if resin composite material is used as restoration.

C. Glass ionomer cement
Glass ionomer cement [GIC] were first introduced in dentistry since 1969. The cytocompatibility and stability over time makes this material to be used in neurologic surgery [fixing ossicular chain prosthesis, cochlear implants and repair of tegmen and posterior canal wall defects]. Screening tests shown that freshly prepared GIC is mildly cytotoxic but its effects reduced over the time. High molecular sized of the polyacrylic acid makes it impossible to diffuse through dentine. Studies shown that GIC appears to be bland to pulp when used as restorative material but induce pulpal response when used as luting agents. The viscosity and hydraulic pressure are attributed to this difference. Light cured GIC [resin modified GIC]has been introduced by including bis-phenol-A-glycidyl methacrylate[BiS-
GMA] or other monomers/oligomers as pendant chains. Any modifications of GIC is shown to be cytotoxic. Several studies demonstrated that Metal modified GIC, Resin modified GIC leaches and diffuse through dentinal tubules and reach dental pulp cells. The magnitude of damage to pulp cells is inversely related to the remaining dentin thickness between the pulp tissue and the material. Stainlawski in 1999 further analyzed the reason for the cytotoxic effects of metal reinforced GIC and confirmed that Zinc along with copper and silver are responsible for cytotoxicity. Lan WH in 2003 compared nine types of GIC cultured on human dental pulp cells and confirmed that resin modified GIC is more toxic than conventional GIC. However in 2003 and 2007, Costa CAS demonstrated with an invivo study in human teeth that resin modified GIC liner didn’t cause any pulpal response in deep class V cavity. In any case GIC or modified GIC’s should not be placed directly on living pulp tissue.

D. Zinc oxide eugenol cement [ZOE]

Both invivo and invitro test proved that eugenol released from zinc oxide cement fixes the cell, depresses cell respiration and reduces nerve transmission upon direct contact. These effects are dose dependent and the magnitude of diffusion through dentinal tubules. It forms temporary seal against bacterial invasion. It cause slight to moderate inflammatory reaction in the first week followed by reparative dentin formation within 5-8 weeks. It has anodyne and obtundant effect on pulp, facilitates pulpal healing but it is irritant on direct contact.

E. Zinc polycarboxylate

Zinc polycarboxylate shows similar pulpal response like ZOE. However reparative dentin formation is very minimal. Concentration of polyacrylic acid above 1% is cytotoxic in invitro test but long term invitro test failed to prove this claim. It was hypothesized that buffering and protein binding might neutralize these effects in long term.

F. Zinc phosphate

It is a combination of zinc and orthophosphoric acid. Invitro tests shown that zinc phosphate cement exhibit strong to moderate cytotoxic effects on pulp but decrease with time. Focal necrosis were seen in test that injected zinc phosphate cement into rat dental pulp. The cytotoxic effects are mainly attributed to its initial low p H [4.2 in 3 min]. Current recommendation is to use a protective liner/varnish beneath the zinc phosphate restoration.
G. Bonding agents
It is well established that dentin is an efficient buffer of protons, as a result most of the acid never reaches the pulp. This also depends on remaining dentine thickness and 0.5mm is considered as an adequate remaining dentin thickness [RDT]. Although acids do not reach pulp invitro studies demonstrated that bonding agents may penetrate up to 0.5 mm into dentine thus causing significant suppression of cellular metabolism for 4 weeks after its application. Smith et al in 1986 confirmed that 40% of orthophosphoric acid remained in tooth even after rinsing. Thus the cytotoxic effects of acid etching and bonding agents on dental pulp depends on following factors: remaining dentin thickness, degree of etching and strength of acid.

H. Liners, varnishes
Generally liners are high alkaline pH (>12) suspension. The high pH leads to extreme cytotoxicity in invitro tests. The initial pulp tissue reaction to this high alkaline pH is the necrosis to a depth of >1mm. within weeks to months those necrotic zone is replaced by dystrophic calcification and act as a stimulus for dentine bridge formation. The high pH provides bactericidal activity and promotes tertiary dentin formation if used as pulp capping agents.

I. Other restorative materials
Metal ions of chromium, cobalt, copper, mercury, tin, nickel and zinc cause cell death /damage in invitro tests. All these metals are used in dental material as base metal alloys, dental amalgam, titanium alloys. However these metals are not found as metal ions in restorations. Further alloying of these metal reduces ion production. The insertion of gold foil may result in pulpal reaction but this is because of condensation force, thermal conductivity, dehydration of dentinal tubules, and micro leakage not because of cytotoxic effects. Conventional dental ceramics are considered to be most inert of all dental material. The pulpal response to inlays, onlays, crowns, bridges are determined by the luting agents used not by the components of casting alloys /ceramics. Almost all luting agents are cytotoxic in invitro tests but with variable differences.

Altering water: powder ratio, viscosity, are some of the measures advised to decrease these cytotoxic effects on dental pulp. Pulpal protection is mandatory if acrylic resin cement is used.
Limitations
The major drawback with classical invitro cytotoxicity test is it does not reflect the long term in vivo behavior of restorative material. Many factors affects the release of components from restorative material such as bacterial metabolite products, water, enzymes, polar and non polar solvents. Current cytotoxicity tests are not exhaustive enough to accommodate all or some of the confounding factors mentioned early. Improving these limitations along with comprehensive database to identify the cytotoxic effects of individual components of commonly used dental materials would be an attractive option for material scientist to design new and effectively redesign existing questionable restorative materials.

Clinical guidelines
Based on the literatures reviewed, the following guidelines are suggested. However it should be followed with caution as it is based on narrative reviews not systematic review or Meta-analysis.

Table 9 Flowchart for clinical guidelines in using restorative biomaterial

7. CONCLUSION
From the literatures eugenol, BiS-GMA, TEGDMA, HEMA, BPA, UDMA, gold, copper, cobalt, mercury, tin, nickel, zinc, chromium, and ortho phosphoric acid are considered to be cytotoxic to dental pulp if placed directly. Acid dental materials have the ability to
demineralize dentin and possibly increases biological effects on dental pulp. Remaining dentin thickness, dentine permeability, duration of exposure influences the cytotoxic effects of the restorative dental material on dental pulp. Tooth slice culture assay shows promising in determining the cytotoxic effects of restorative materials pertain to dental pulp. With the revolution of new materials around, immortalized odontoblasts, developing new cell lines, three dimensional tooth slice culture, are some of the interesting avenues to be explored to improve the reliability of current invivo cytotoxicity tests. Despite various limitations, current invivo cytotoxicity tests guided us in providing materials for safe clinical use. Knowing the cytotoxic effects of each components helps the clinician in proper decision making to choose appropriate pulp friendly restorative material.

REFERENCES

REFERENCES

A. Included articles


Excluded Articles [ N= 84]

A. No information on cytotoxic effects, on dental pulp/ Cytotoxicity of bleaching/whitening.


B. Endodontic Perspective.


C. Non English.


D. Original research, Technical reports, Letter to editor, short communication, Non indexed.


5. Chang, M.-C., et al. "The role of reactive oxygen species and hemeoxygenase- expression in the cytotoxicity, cell cycle alteration and apoptosis of dental pulp cells induced by BisGMA." Biomaterials 2010; 31(32): 8164-8171


