Histopathological picture of heat cured acrylic resin modified with nano-composite filler (in vivo study)

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ABSTRACT:
Heat cured polymethyl methacrylate is a biomaterial with a cytotoxic effect modified by composite fillers to improve its mechanical properties need to evaluate tissue reaction to the modified materials.

Material & Method
A 45 heat cured acrylic disc specimens were divided into control group which is acrylic resin specimens without reinforcement, and test group specimens reinforced with the silanated Aluminum silicate nanoparticle powder fillers in a concentration ratio is 3% and 5%. These specimens were implanted in a 15 Wister rats. The implanted areas undergo biopsy after 3 days, 14 days, and 4 weeks. Then, prepared for histopathological examination.

Results
Within the limitation of this study the results revealed that the control groups induce more tissue reaction and show a significant inflammatory cell count as compared to modified test groups in all observation periods

Conclusion
Modification of heat cured acrylic denture base resin with alumino silicate nano particles in concentrations 3% and 5% consider more biocompatible than unmodified groups.

Keyword: acrylic, nanoparticles, biopsy, host response, in vivo study


INTRODUCTION:
Biomaterials should function well with appropriate host response. Materials that are biocompatible in contact with the oral mucosal surface may cause adverse reactions if they are in contact with underlying tissue. (1) The cytotoxic effect of Polymethyl methacrylate polymer has been proved. (2) Due to its residual monomer content (3), or other toxic eluent materials (4). Poly methyl methacrylate denture base material has widely used for denture base, maxillofacial prostheses and in many dental restorative procedure. It has direct and indirect contact with deep parts of hard and soft living tissue. It has been revealed that increase the level of pro inflammatory salivary cytokines (TNF-α & IL-6) in denture wearers from the new denture delivery which declined with time. (5) The broad application of polymethyl methacrylate necessitates improvement of this material either by changing mode of polymerization (2) or addition of fillers (6) or replacing the material with another polymers.(7,8) Incorporation of inorganic composite fillers have been widely investigated as heat cured acrylic denture base reinforcement for both mechanical and
physical properties(6). The employment of Nano technology in manufacturing fillers modified acrylic denture base materials have been broaden the area of polymer enhancement (6,9) compared to composite microfillers (10). Mixing of polymethyl methacrylate with various types of reinforcing fillers resulting in new materials that effectively improved its mechanical and physical properties than control group, so introduction of such new material to be in use like the original material necessitates the knowledge of its biological effect on the living tissue they act upon. Biological testing varies from cellular response obtained by cell culture test called the primary tests, secondary animal tests to measure tissue responses, and usage tests in large animals. All or part of these tests must be conducted before the decision for such a material being applied in humans. (1, 11) The subcutaneous implanted material was applied in small animals like rats, rabbits, or other animals to reflect the living system reaction to acrylic denture base materials with fillers consider acceptable(12,13) heat cured acrylic materials cause an acute inflammatory reaction which become chronic over time as shown from The subcutaneous and intramuscular implantation of the acrylic samples in rats.(14) Aluminum silicate composite as a filler can enhance the mechanical properties of heat cured acrylic resin(15) as well as proved its biocompatibility.(16) The present study used to determine the rats subcutaneous tissue reaction to the effect of heat cured acrylic resin reinforced with 3% and 5% by weight aluminum silicate nano filler.

2. MATERIALS& METHODS:

2.1 Surface treatment of aluminosilicate (Al2SiO5R) nano-particles with (3-methacryloylpropyl) trimethoxysilane (MPS) according to (17)

2.2 Specimens’ grouping: A 45 heat cured acrylic disc specimens (Vertext, Netherland). 15 specimens were acrylic resin without reinforcement considered as control group. The remaining 30 specimens were equally divided into two test specimen groups reinforced with the silanatedAlumium silicate nanoparticle powder fillers (The British Drug Houses LTD.B.D.H. Laberatory Chemical Group Poole England). The fillers concentration ratio is 3% and 5%.

2.3 Specimen preparation: All specimens were prepared in dimensions of 5mm diameter and 2mm thickness, obtained from brass discs imprint in putty condensation silicon (Zermach, Italy) placed in stone to get disc mold for compressed dough acrylic resin (a). With aid of an electronic balance with accuracy of (0.0001g) the silanatedAlumium silicate powder fillers weight were estimated, the fillers are added and well dispersed to the monomer, using a probe sonication apparatus (120 W, 60 Khz) for 3 minutes to break them into individual nano crystals.(b) Then, quickly mixed with the heat cured powder in ratio used in this study 6g/3ml powder/ liquid ratio. The conventional flasking method for complete denture preparation was followed. The specimens were cured using short curing cycle. After that, all specimens were finished and sterilized in sodium hypochlorite (0.5%) for 10min (c).then, the specimens conditioned in distilled water for 24 hours at room temperature.

2.4 Animals grouping: a 15 young male rats five months old, and mean weight of 200-250g were used. During the experimental period the animals were kept in animal house belong to college of pharmacy in Karbala university. The animals were grouped into 3 groups corresponds to the experimental times (3 days, 2 weeks, and 4weeks). Each group consists of 5 animals.

2.5 Surgical procedure: The rats were anesthetized by intramuscular injection 0.1ml/kg ketamin HCL mixed with xylezine hydrochloride 0.05ml/kg. The fur skin was shaved manually over the back. The shaved area was cleaned with idoform antiseptic solution, and then was divided by vertebral column into right and left side. The control specimen was implanted in the right side. The test specimen contains 3% silanated aluminum silicate filler implanted on the right side about 2cm below the control specimen. In the left side, the 5% silanated aluminum silicate filler test specimen was implanted. The subcutaneous supramuscular tissues were separated with blunt end scissors to create a pouch for the specimen placement then suture the poach edges. At the end of each time intervals (3days, 14 days, 30days) the rats anesthetized and shaved over the accused area. the specimens with the surrounding tissues including skin and subcutaneous tissues were cut with scalpel the specimens were removed and the specimens biopsy kept in10% formalin. All the specimens were submitted for routine hematoxyline-eosin histopathological process.

RESULTS:
This is a prospective mode of study. It implies the usage of an experimental animal (rat) to show the histopathological effect of polymethyl methacrylate before and after modification with 3% and 5% aluminum silicate filler in frequent intervals 3 days, 2 weeks and 5 weeks for the three studied groups (the control, the 3% and the 5% modified specimens).

**Table 1: Lymphocytic infiltration (per high power field)**

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL</th>
<th>3%</th>
<th>5%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAYS</td>
<td>40</td>
<td>21</td>
<td>12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2 WEEKS</td>
<td>100</td>
<td>22</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>4 WEEKS</td>
<td>30</td>
<td>7</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

The above table illustrates the chronic inflammatory cells (lymphocytes) in the 3 groups of the study. The data demonstrate that the usage of the pure polymethyl methacrylate induces more inflammation while modification with the 5% aluminum silicate was associated with least inflammation with a significant difference among the groups (<0.05) seen in figure 1, 2, 3&4. Acute inflammation with tissue neutrophil infiltration can be a tissue response against foreign substances. This fact was clearer with the usage of the pure polymethyl compound especially after 3 days duration while the No. was reduced significantly with the modification and adding aluminum filler especially the 5% concentration. This information can be seen in the below table and figure 1&2.

**Table 2: Neutrophil infiltration in the upper dermis (per high power field)**

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL</th>
<th>3%</th>
<th>5%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAYS</td>
<td>120</td>
<td>75</td>
<td>13</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2 WEEKS</td>
<td>20</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4 WEEKS</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Fibroblast cells responsible for laying down the elastic and the collagen fibers, replicate more when there is a damage to the parenchyma of the subcutaneous tissue, so this type of cells are more associated with the chronic inflammation. This fact was seen more in the control group especially with the 2 weeks duration. About similar proliferation of this type of cells was watched in the other groups, and with no statistically significant difference (p value > 0.05) as seen in the below table.

**Table 3: Fibroblast cells in mid-dermis (per high power field)**

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL</th>
<th>3%</th>
<th>5%</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAYS</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2 WEEKS</td>
<td>30</td>
<td>25</td>
<td>24</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>4 WEEKS</td>
<td>22</td>
<td>16</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>
Epidermis can be affected due to the underlying inflammation. The 2 weeks sample of the control group was associated with the highest epidermal layers number which can be correlated with the bulk of inflammation, but with no significant difference with the other groups (p >0.05) as shown in the table.

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL</th>
<th>3 %</th>
<th>5%</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAYS</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2 WEEKS</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4 WEEKS</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td></td>
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</table>

Ill-defined foreign body granulomas were formed in the control cases especially after 2 and 4 weeks duration while this histological structure was not seen with the admixed resin whether the 3% or the 5% concentration, demonstrated in figure 5. Collagen deposition was more seen in the control group (especially the 2 week nos duration), with about similar proportion of collagen lying down for both the 3% and the 5% concentration groups as seen in figure 3.

Figure 1: control group: After 3 days duration, showing intense inflammation (mixed acute & chronic cells) in the upper dermis & epidermis (10x power).

Figure 2: control group 4 weeks duration, still there is an active inflammation in the upper dermis with mild atrophied epidermis (10x)
DISCUSSION:

This study investigates for the biological response to the heat cured acrylic denture base resin after modifying with composite nano fillers. The cytotoxic and unwanted effect of the resins as a prosthetic materials was studied both in vivo and in vitro (21, 22). The usage of experimental animals like rats is a relatively effective in vivo method of studying the histopathological changes associated with implanting of biomaterials (23, 24). Acute inflammation can be a tissue response against foreign substances. After 3 days duration, this fact was more clear with the non-treated acrylic resin control group. This fact was declared by viewing the increasing number of the acute inflammatory cells. The number of such cells were reduced significantly with the applying of modified reinforced specimens as shown in table 1 &2 and figure 1 Regarding to the 3% treated specimens biopsy there was less inflammation and granulation tissue to the non-treated acrylic resin material (control), epidermis shows less inflammatory reaction, no granuloma formation, and the tissue response was improved significantly over time compared to the control group. While, the least inflammatory reactions and granulation tissue with no significant epidermal changes, no granuloma formation and mild collagen laying down was observed in the 5% modified resin which is also improved over time in
comparison with the control group (Table 3 & 4). The preliminary granuloma was seen only in the control groups as seen in the Figure 5. This is explained by the fact that the granuloma can occur only in some types of prosthodontic materials due to tissue irritation while in other components may be minimal or absent according to the ability the dermally implanted materials (25, 26). The histopathological examination shows that applying of non-modified resin materials is associated with more inflammatory response, granuloma (figure 3) formation and fibroblast proliferation (table 3) as compared to the treated 3% and 5% specimens. Aluminum silicate fillers replace part of polymer in a percentage 3% and 5%, as a result, the amount of polymethyl methacrylate is less in the test groups compared to control group which subsequently leads to decrease the amount of residual monomer in test groups as declared by the reduction in the inflammatory features in test groups compared to control groups this results agreed with previous studies (27, 28) The var in the tissue response of the three groups is attributed to the differences in the chemical composition of the three different implanted groups, in other ward the eluted chemical compounds. The main harmful leachable compound is the residual unreacted monomer methyl methacrylate may cause that can lead to allergic reaction, chemical irritation, or toxic side effects for both clinicians and the patients. (3, 29, 30) With the usage of standardized method of processing, The amount of residual monomer can be governed by the curing process and powder/liquid ratio of the acrylic denture base. (11) The previous studies revealed that the presence of unreacted residual monomer in denture base acrylic resins is unavoidable in the heat cured acrylic denture base irrespective of the curing technique used (3, 31).

CONCLUSION:

Reinforcement of heat cured acrylic denture base polymethylmethacrylate resin with 3% and 5% aluminosilicate nanocomposite fillers reveal more biological acceptance with least subcutaneous tissue inflammation with 5% concentration group.

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