Evaluation the biological effect of two types of denture base materials reinforced with silanated glass fiber

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ABSTRACT
Background: Many attempts to improve the mechanical properties of acrylic denture base materials by adding filler such as silanated glass fiber (SGF). This new product (acrylic resin + SGF) need to evaluate its biocompatibility because of its application in contact with the living tissue. The aim of this study was to evaluate the Biological Effect of two types of denture base materials (heat-cured, light-cured) reinforced with randomly oriented short (SGF) at four different times.

Materials and Methods: Specimens of heat and light cured denture base materials reinforced with (2mm length, 2wt %) (SGF) was implanted in the subcutaneous tissue at the back of the Newland rabbits. Biopsies for histopathological observation were taken after (3days, 7days, 14days, and 30days).

Results: Histopathological observation showed mild to moderate inflammation in the subcutaneous tissue of the rabbits, and tissue acceptance improved over times.

Conclusion: It was noted that silanated glass fiber is a biocompatible material when added to heat-cured and light-cured resins. Both resins demonstrated good level of biocompatibility within the connective tissue of the rabbit.


INTRODUCTION
The development of dental materials is growing every year, as well as their use. Among the dental materials, acrylic resins have important application, mainly in dental and maxillofacial prosthesis. The material need to be exhaustively studied in order to get better knowledge of their biological, physical and chemical properties. This is particularly important when it is considered that these materials can be put in direct contact with the living tissues. In this case, Biocompatibility is the goal to be reached (1,2).

Polymethylmethacrylate resin used in removable dental prosthesis has the potential to elicit irritation, inflammation and allergic reaction to the oral mucosa (3,4, and 5).

There are some types of material that added to acrylic resin in order to improve its mechanical properties. Such materials are the addition of rubber graft copolymer or reinforcement of polymethylmethacrylate with other materials such as carbon fiber, glass fiber, aramid fiber, ultra high modulus polyethylene, and metal insert (6). Glass fiber reinforced polymers enhance the mechanical properties of the polymers especially the silanated glass fiber (SGF) because of their good initial bonding between the glass fiber and polymers via the interface that made from silane coupling agent (7). However, few studies evaluated cytotoxicity of fiber-reinforced acrylic resin denture in vitro such as glass fiber and carbon fiber (8,9). The present study used to determine the biological effect of glass fiber reinforcement of two types of denture base material heat-cured and light-cured resins. That performed by subcutaneous implantation in the tissue of the back of Newsland rabbits.

MATERIALS AND METHODS
A 40 adult white rabbits (Newsland) weighing 1-2 Kg were grouped into 20 rabbits for heat cured specimens and 20 rabbits for light cured specimens. Each 20 rabbits divided into 4 groups, which correspond to the experimental times. Each group consists of 5 rabbits. Both heat cured resin (Dentsply, Stellon QC-20, England) and light cured (Megadenta, Germany) were used to make 80 discs with 6mm in diameter and 2mm thickness (10). A 40 discs represented the control which is denture base materials without SGF, and 40 discs represented the test which is denture base materials with (2mm length, 2wt %) SGF. The heat-cured acrylic was mixed in a ratio used in this study 7.5mg/4ml powder/ liquid ratio. All specimens were finished and sterilized in sodium hypochlorite (0.5%) for 10min (11). After that, the specimens conditioned in distilled water for 24 hours at room temperature. Rabbits were anesthetized by intramuscular injection 1ml/kg ketamin HCL mixed with xylezine hydrochloride 0.1ml/kg. The fur skin was shaved manually over the lower part of the back. The shaved area was divided by vertebral column into right and left side. The control specimen was implanted in the right side. In the left side, the test specimen was implanted. An incision of 1cm length was made through the skin. The subcutaneous supra muscular tissues were separated with blunt end instrument to create a pouch for the specimen.
Then the specimen was held with a pair of tweezers and inserted in the pouch, 5mm away from the incision line\(^{(12)}\).

The incisions were sutured and the area was cleaned and disinfected with Savelon disinfectant solution. After 3, 7, 14, and 30 days of the implantation periods the rabbits were anesthetized again. A 2 cm excisional biopsy was taken, which involved the skin, the embedded specimen with some of supra muscular tissue. This biopsy placed in 10% buffered formalin for one-day fixation. The specimens were removed and the tissues were conventionally processed (paraffin inclusion), and stained by haematoxylin and eosin (H.E), for light microscopical observation.

RESULTS

The results of clinical findings revealed that all subcutaneous implant sites appeared to heal satisfactory at all intervals.

The tissue supporting specimens of heat-cured resin without SGF showed moderate inflammation, presence of plasma cell (PC) and macrophage (MC) after 3 days of implantation. After 7 days, the inflammatory reaction tended to subsided to a certain degree but still moderate inflammation with presence of polymorphonuclear cell (PMN). Healing progressed with some chronic inflammatory cells present at 14 days. A well-organized fibrous capsule formed after 30 days. As shown in figure (1).

In case of histopathological examination of biopsy involved heat-cured acrylic reinforced with SGF specimens. At 3 days showed loose connective tissue that became a well-organized fibrotic capsule at 7 days. After 14 days became a band of fibrotic capsule, the healing improved with a wavy coarse collagen fibers forming capsule at 30 days. As shown in figure (2).

Microscopical field of sections obtained from biopsies contained light-cured acrylic without SGF revealed severe inflammatory reaction after 3days of implantation. This reaction became moderate at 7 days of implantation at this period there was evidence of irregular loose connective tissue which became thin capsule after 14 days with presence of chronic inflammatory cell. Then healing improved with the formation of thin fibrous capsule at 30 days. As shown in figure (3).

In case of histopathological examination of sections obtained from biopsies contained light-cured acrylic resin reinforced with SGF specimens the results showed at 3 days a loose cracked connective tissue. At 7 days presence of continuous connective tissue the type of inflammation is a moderate one with presence of lymphocyte cell (LC), this connective tissue became a band of fibrotic capsule at 14 days. The healing improved with formation of thick compact fibrotic capsule after 30 days. As shown in figure (4).

DISCUSSION

The use of short cutting randomly oriented SGF in denture base found to be simple technique\(^{(13, 14)}\). Also the use of 2mm length SGF and 2% by weight was proved to improve the mechanical properties of heat-cured acrylic\(^{(14)}\). The powder liquid ratio (7.5mg /4ml) was used for heat-cured resin groups reinforced with SGF and without SGF for a good impregnation of GF in the polymer.

In this study, it was used subcutaneous implantation test. Standardized subcutaneous implantation method has proved an efficient and reproducible for revealing differences in the biological performance of dental material\(^{(15)}\). The surgical trauma and foreign body introduced could be the cause of reaction after 3 days of implantation for all groups. It was proved that the fibrous capsule formation is an indication of good tissue reaction to the implanted materials because fibrous capsule formation separates foreign material from other tissue to minimize damage\(^{(16)}\).

In case of histopathological examination of biopsy involved heat-cured acrylic reinforced with SGF specimens, it showed that the fibrous capsule formed after 7 days while the section include the heat-cured only the fibrous capsule formed after 14 days. Heat-cured acrylic resin reinforced with SGF specimens showed less tissue reaction in all periods of time conducted in this study in comparison to biopsies involved heat-cured only. These results agreed with Vallittu and Ekstrand\(^{(17)}\), and Meric et al\(^{(9)}\) who evaluated the cytotoxicity of heat-cured denture base materials reinforced with SGF, they concluded that SGF reinforced heat-cured denture base material was free of cytotoxicity as analyzed by invitro cytotoxicity test. Also agreed with Sipahi et al\(^{(18)}\). The later showed that polymers reinforced with silane treated GF had lower cytotoxic effect than polymers reinforced with monomer treated GF.

As a result, they assumed that the cytotoxic effect of fiber reinforced polymers is not inherent from the fibers but from the impregnation method of the fibers into the acrylic resin bulk. Since the powder to liquid ratio was standard in this study, the explanation of less toxicity in heat-cured denture base material reinforced with SGF due to the addition of SGF to the polymer, which lead to reduce the amount of polymer in comparison to the polymer without SGF. So reduce the amount...
of leachable toxic component in reinforced groups than unreinforced group.

In case of histopathological examination of sections obtained from biopsies contained light-cured acrylic resin, the results had better biocompatibility for reinforced specimens as compared with unreinforced group, since the reinforced group exhibited less tissue reaction and thicker fibrous capsule formation than unreinforced group. These results supported by Vakiparta et al (18) and Shinya et al (19) who concluded that the GF in composite affect the polymerization and the degree of conversion (DC %) So the results of this study could be explained by that. The addition of SGF to light-cured denture base materials increased the conversion of monomer to polymer, which leads to increase the biocompatibility of the material due to decrease in leachable toxic component that stimulate tissue reaction.

Generally, the histopathological observations revealed a good level of biocompatibility for both types of resins. In the studied model, slight differences were observed as the light-cured resin demonstrated less biocompatible reaction compared with heat-cured resin in different times studied. These results agreed with AL-Aani (12).

It was suggested that the light-cured denture resins release multiple toxic components that can produce different cell response invitro (1). Thus, the explanation why light-cured resin induced more tissue reaction than heat-cured resin could be because of presence of toxic component in light-cured resin that had higher degree of cytotoxicity than heat-cured resin.

REFERENCES

Figure 1: Heat cured acrylic control group

Figure 2: Heat cured acrylic test group
3 days severe inflammation

7 days irregular loose connective tissue

14 days thin layer capsule

30 days thin sheath capsule

Figure 3: Light cured acrylic control group

3 days loose cracked connective tissue

7 days moderate inflammation with lymphocyte cell LC

14 days band of fibrotic capsule

30 days thick compact fibrotic capsule

Figure 4: Light cured acrylic test group