X-ray diffraction and biocompatibility of glass ionomer cement reinforced by different ratios of synthetic hydroxyapatite

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ABSTRACT

Background: This study was done to assist X-ray diffraction and biocompatability of glass ionomer cement reinforced by different ratios of Hydroxyapatite.

Materials and Methods: The powder of glass ionomer cement reinforced by different ratios of Hydroxyapatite were used to get X-ray diffraction pattern by X-ray diffraction machine, While for biocompatibility test, A polyethylene tubes containing glass ionomer cement reinforced by different ratios of Hydroxyapatite were implanted on the dorsal submucosal site of Rabbit's tissues and histological slide were prepared for histopathological study.

Results: X-ray diffraction test showed that all elements of glass ionomer cement reinforced by different ratios of Hydroxyapatite were react with each other and all the final products none crystalline in nature with small amount of Hydroxyapatite present unreacted may be act as cores for final reacted elements. The histological test showed mild irritation to Rabbit's tissues by glass ionomer cement reinforced by different ratios of Hydroxyapatite, this irritation subsided with time.

Conclusions: there is chemical reaction of all elements of glass ionomer cement reinforced by different ratios of Hydroxyapatite and new final products were results .Also glass ionomer cements reinforced by different ratios of Hydroxyapatite were biocompatible with Rabbit's tissues.

Key words: X -ray diffraction, Biocompatibility, glass ionomer cement. (J Bagh Coll Dentistry 2013; 25(3):62-68).

INTRODUCTION

Glass ionomer cement was first introduced to the dental profession by Wilson and Kent in 1972. Their main characteristics are an ability to chemically bond to enamel and dentine with insignificant heat formation or shrinkage; Biocompatibility with the pulp and periodental tissues, fluoride release producing a cariostatic and antimicrobial action, less volumetric setting contraction and a similar coefficient of thermal expansion to tooth structure. These advantages have made them successful as luting cement and lining materials. However, as a restorative material, their sensitivity to moisture and low mechanical strength and wear resistance make them the least durable. This may be adequate for primary teeth because they will exfoliate in a number of years.[1,2]

When the powder and liquid in conventional glass ionomer are mixed together, an acid-base reaction occurs between the polyalkenoate acid and ion leachable glass, resulting in a plastic paste which then hardens to a sold mass. The final set structure is a complex composite of the original glass particles sheathed by a siliceous hydrogel and bonded together by a matrix phase of hydrated fluoridated calcium and aluminum polyacrylate .[3, 4] While GICs have been used successfully for over 30 years in dentistry, there are still concerns regarding GIC biocompatibility in non-dental applications.

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In particular, Al^{3+} release has been associated with poor local bone mineralization and local neurotoxicity .[5]

Numerous cell culture studies of cytotoxicity have reported cell inhibition by specific GIC compositions, and Brook and Hatton reviewed this work in 1998 .[6] It appeared that the in vitro toxicity of GICs was due to a complex mechanism based on both ion release (in particular, Al^{3+} and F^-) and pH effects .[7] Early animal studies of GIC bone cements provided evidence of good biocompatibility. [8]

In this study we assist the X-ray diffraction of glass ionomer cement reinforced by different ratios of Hydroxyapatite and its biocompatability with Rabbit's tissues.

MATERIALS AND METHODS

Preparation of experimental cement:

A powder of glass ionomer cement (medicem; Promedica; neumonster; Germany) was mixed with different ratios (10%, 15%, 20%, 25%, 30% by weight) of Hydroxyapatite then the mixed powder was applied in agate machine for half an hour and hour for grinding and to get homogenous mixture (better distribution of Hydroxyapatite particles throughout the glass ionomer powder) while the liquid of glass ionomer cement remained unchanged.

X-ray diffraction test:

A sample of experimental materials were prepared to undergo this test by mixing the

experimental powder and liquid materials and preparation of a cylindrical specimen by using a stainless steel mould constructed according to ADA specification No.6 then the sample were pulverized and exposed to CuKα radiation by using Mn filter, the values of diffraction will be measured and spacing will be calculated according to Bragg`s equation:

$2d=\lambda / \sin\theta$

Where: d: spacing (distance), λ : is the wavelength of CuKα radiation

θ: is the diffraction angle

Using the value 1.54Aº for the wavelength of CuKα radiation, the figures of spacing (d) will be calculated and compared with ASTM standard table to detect the crystal components in the set materials.

Histopathological study:

Biocompatibility test was carried out on the rabbits by submucosal implantation of the control group (glass ionomer cement) and experimental groups {glass ionomer cement reinforced by different rations of Hydroxyapatite only (10%, 20% and 30% was used for this test)} by using polyethylene tube as a carrier. Females rabbits weight 2-2.5Kg was used in this study. The rabbits were divided into three groups according to period of implantation of the polyethyline tubes (3, 10 and 21 days). Each rabbit has received two implants submucosal on the dorsal position (in order not to get trauma on the implanted site during movement of the rabbits).

Anesthesia:

General anesthesia was performed by intramuscular injection of a mixture of 88mg/Kg of body weight of Ketamine chloride (50gm/ml) and 10 mg/Kg body weight of Xylazin (2%).

Sterilization:

The sterilization of the polyethylene tube was performed as follows

a- The tubes were immersed in pure ethyl alcohol for 15 minutes.

b- Then the tubes were washed with normal saline solution.

c- Autoclaving the tubes for half an hour at 1000C was the last step of sterilization the polyethylene tube.

All the instruments that were in contact with the rabbits were pre-sterilized by autoclaving.

Preparation of the implants:

The polyethylene tubes of 0.5mm internal diameter and 1mm external diameter were loaded with cement materials after mixing , and cut to pieces of 5mm in length(a negative pressure were used to easy the loading the base materials by sucking the other end of the tube by manual sucker).

Preparation of the implanted site:

The rabbit was anesthetized by intramuscular injections then the anesthetized rabbit was placed on its abdominal side on a sterilized surgical board. The dorsal skin of the intended implantation area was shaved and disinfected by povidone iodine solution, the area was located 5-6 cm from the center of the dorsal side toward the tail, where it was observed to be the most difficult place to be scratched by the animal itself.

Implantation of the base materials:

Under the aseptic conditions, two incisions of approximately 10mm in length were made through the skin, one on each side of the dorsal side. The submucosal tissue was opened by blunt dissection, then the Polyethelene tubes filled with cement materials were held from the middle by a straight tweezers and inserted at the implanted site at least 2cm from the line of the incision after the implantation of each tube the wounds were sutured and the skin was scrubbed again with povidon iodion disinfectant.

Animals grouping:

The rabbits were grouped for three time intervals of three days, ten days and three weeks (21 days).

Euthanasia:

The rabbits of a time period were sacrified, each rabbit was injected intra-muscular with large dose of anesthetic Ketamine chloride and Xylesin.

Preparation of histological section:

The tubes were removed with the tissue, which was cut out in rectangular pieces to facilitate directional embedding and correct sectioning, then were immersed in 10% buffered formalin. The tissue was fixed and processed for Parafin wax embedding, serial sections were cut to a thickness of 8μm by a microtome. One of every 10 slices was taken and placed in a water bath and then placed on a slide, which was taken to an oven at 40^{o}C to adhere the slice to the slide. The slide was placed in Xylol to remove the paraffin surrounding the tissue. The slide was placed in a bath containing Haematoxelin and Eosin stain and was left for 10 minutes to stain the tissue. The slide was removed from staining bath, rinsed with distilled water and a glass cover was luted on the stained tissue with Canada balsam.

Slides examination:

Each slide was examined under light microscope at magnification of 12.5X and 20X to evaluate the intensity and degree of inflammatory reactions around each tube end, and the subsequent tissue healing at the sites of implantation. Slides were examined with aid of specialist in oral pathology, using double blind technique.

RESULTS

A- **X-ray Diffraction test:**

X-ray diffraction test was performed on the following

1- For the Hydroxyapatite, powder of glass ionomer cement, liquid of glass ionomer: The x-ray patterns showed in figure(1, 2, 3).The results showed that both Hydroxyapatite and glass ionomer powder have crystalline structure while the liquid of glass ionomer cement has no crystalline structure because it composed of inorganic acids.

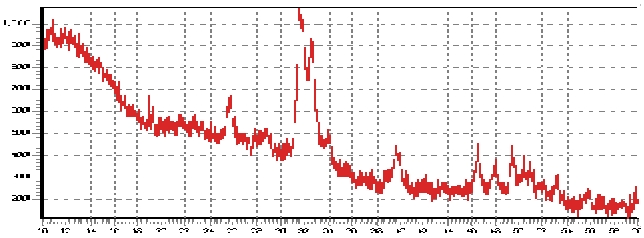

Figure 1. x-ray diffraction pattern of synthetic Hydroxyapatite.

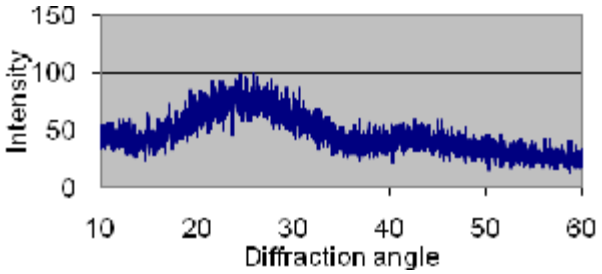

Figure 2. x-ray diffraction pattern of powder of glass ionomer cement.

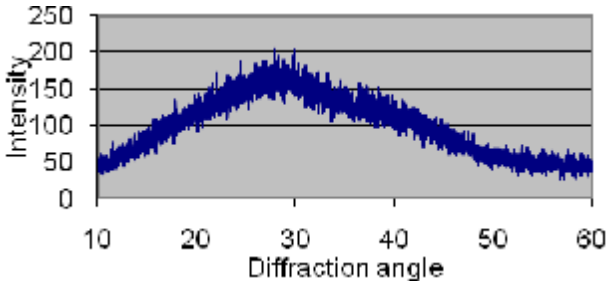

Figure 3. x-ray diffraction pattern of liquid of glass ionomer cement.

2-For the powder of set glass ionomer cement material figure (4): The results of this test showed that the final set material of glass ionomer cement has crystal of unreacted ZnO, may be act as cores for the set material.

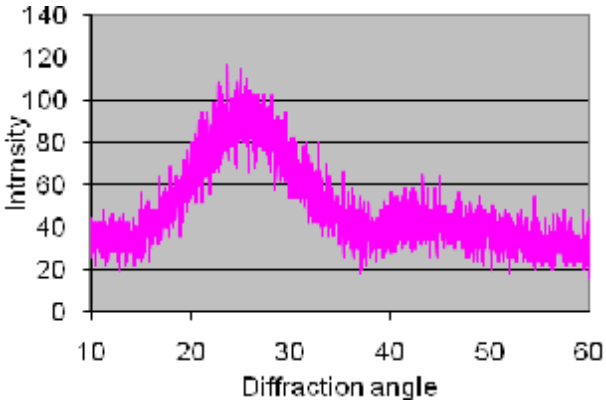

Figure 4. x-ray diffraction pattern of set glass ionomer cement.

3-For the powder of set glass ionomer reinforced by (10%, 15%, 20 %, 25% and 30%) cement materials figure (5 ,6 ,7, 8 ,9):

The results of this test showed that the final set material of glass ionomer cement reinforced by different ratios of Hydroxyapatite has crystals of unreacted ZnO and Hydroxyapatite may be act as cores for the set material while other products of setting reaction non crystalline in nature also the results showed that most of Hydroxyapatite particles shear in the setting reactions giving new products non crystalline in nature.

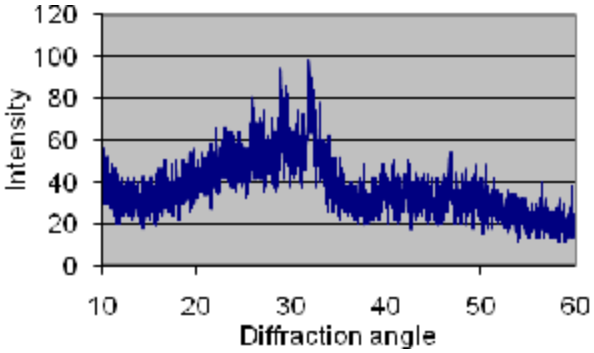

Figure 5. x-ray diffraction pattern of set glass ionomer cement reinforced by 10% Hydroxyapatite.

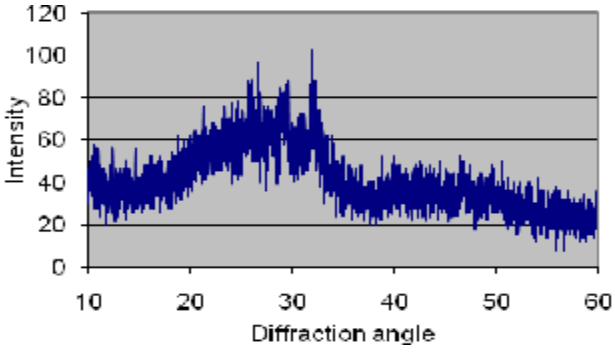

Figure 6. x-ray diffraction pattern of set glass ionomer cement reinforced by 15% Hydroxyapatite.

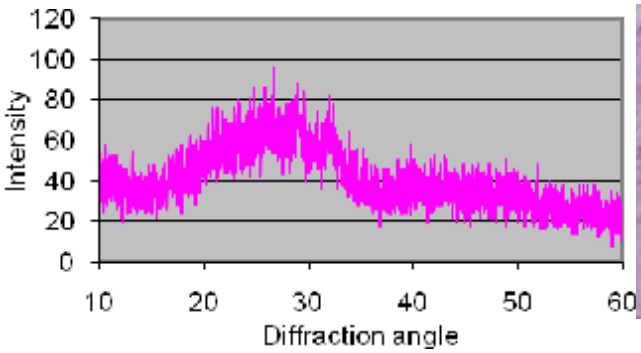

Figure 7. x-ray diffraction pattern of set glass ionomer cement reinforced by 20% Hydroxyapatite.

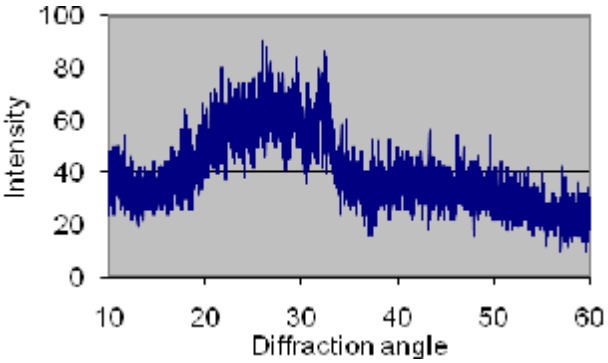

Figure 8. x-ray diffraction pattern of set glass ionomer cement reinforced by 25% Hydroxyapatite.

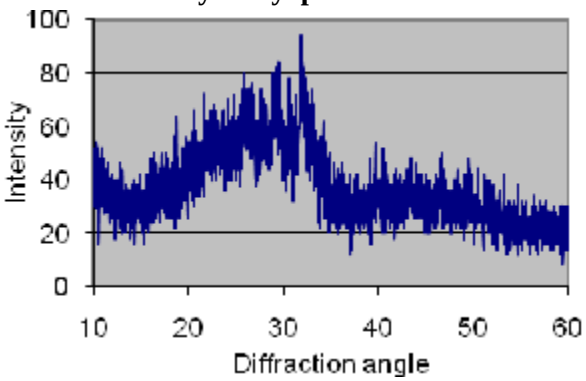

Figure 9. x-ray diffraction pattern of set glass ionomer cement reinforced by 30% Hydroxyapatite.

B-Histopathological test:

The histopathological pictures were qualitatively examined under light microscope regarding the intensity of the inflammatory response of the rabbit's submucosal tissues to the implanted controls and experimental groups and the degree of the subsequent tissue healing at different time periods.

1-For the glass ionomer cement:

At three days: Sever inflammatory response extend to the lateral side of the tube with necrosis near the implanted material figure(10).

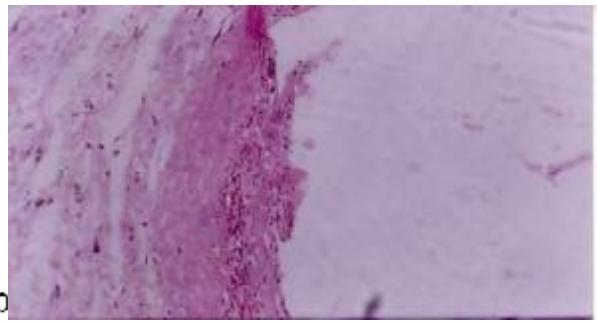

Figure 10. Polyethylene tube filled glass ionomer cement after 3 days Hematoxylin and Eosin.X 20.

At ten days: There is large mass of granulation tissue figure (11).

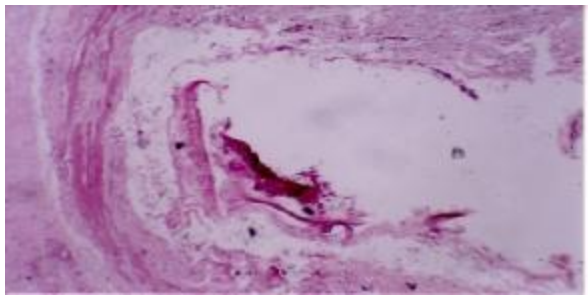

Figure 11. Polyethylene tube filled glass ionomer cement after 10 days Hematoxylin and Eosin.X 20.

At three weeks: there was connective tissue, hyalinization, fibrous tissue appeared in large area as hallow around the end of the implanted tube. There was coagulation degeneration adjacent to the base material figure (12).

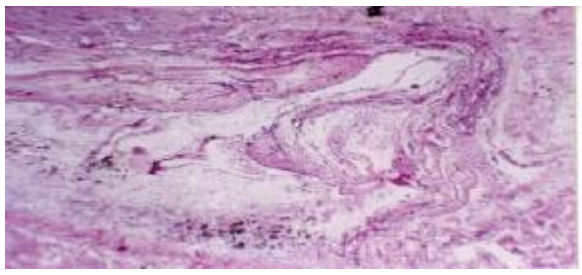

Figure 12. Polyethylene tube filled glass ionomer cement after 3 weeks Hematoxylin and Eosin.X 20.

2-For the glass ionomer cement reinforced by 10% Hydroxyapatite:

At three days:

Sever inflammatory response extend to the lateral side of the tube with necrosis near the implanted material figure (13).

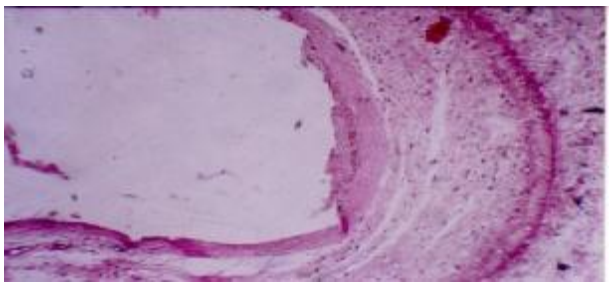

Figure13. Polyethylene tube filled glass ionomer cement reinforced by 10% Hydroxyapatite after 3 days Hematoxylin and Eosin.X 20.

At ten days: There is large mass of granulation tissue figure (14).

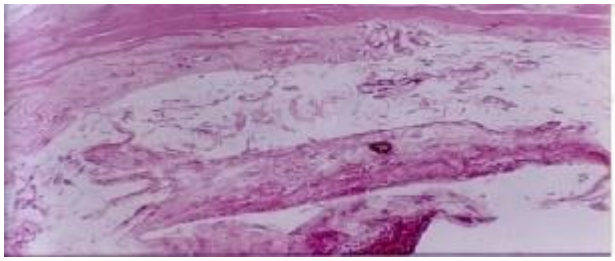

Figure 14. Polyethylene tube filled glass ionomer cement reinforced by 10% Hydroxyapatite after 10 days Hematoxylin and Eosin.X 20

At three weeks: there was connective tissue, hyalinization, fibrous tissue appeared in large area as hallow around the end of the implanted tube. There was coagulation degeneration adjacent to the base material figure (15).

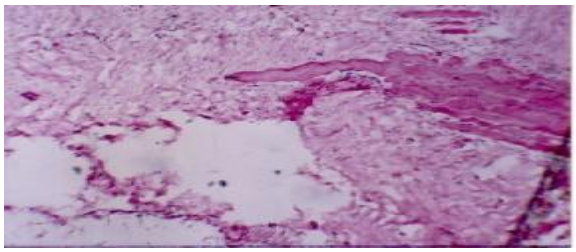

Figure 15. Polyethylene tube filled glass ionomer cement reinforced by 10% Hydroxyapatite after 3 weeks Hematoxylin and Eosin.X 20.

3-For the glass ionomer cement reinforced by 20% Hydroxyapatite:

At three days: A cute inflammatory cells mainly neutrophiles and there was fibrous tissue figure(16).

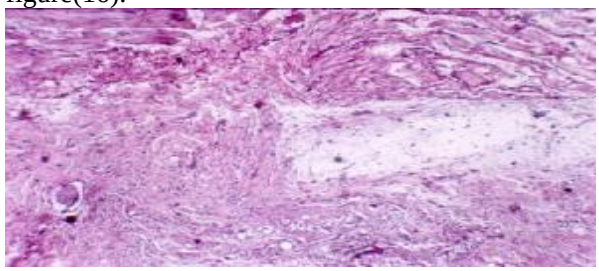

Figure 16. Polyethylene tube filled glass ionomer cement reinforced by 20% Hydroxyapatite after 3 days Hematoxylin and Eosin.X 20.

At ten days: Thick dense fibrous tissue with mature fibroblast cells figure (17).

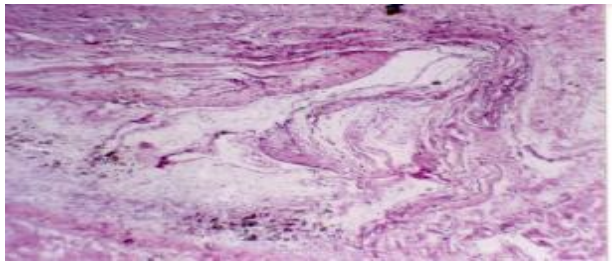

Figure 17. Polyethylene tube filled glass ionomer cement reinforced by 20% Hydroxyapatite after 10 days Hematoxylin and Eosin.X 20.

At three weeks: A small mass of loose fibrous tissue with large active fibroblast cells (no inflammation) figure (18).

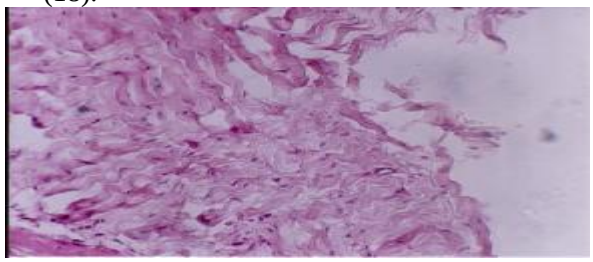

Figure18. Polyethylene tube filled glass ionomer cement reinforced by 20% Hydroxyapatite after 3 weeks Hematoxylin and Eosin.X 20.

4-For the glass ionomer cement reinforced by 30% Hydroxyapatite:

At three days: A cute inflammatory cells mainly neutrophiles and there was fibrous tissue

figure(19).

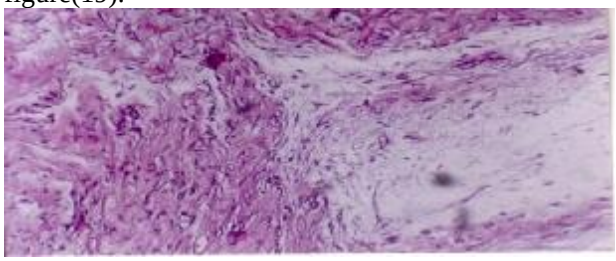

Figure 19. Polyethylene tube filled glass ionomer cement reinforced by 30% Hydroxyapatite after 3 days Hematoxylin and Eosin.X 20.

At ten days: Thick dense fibrous tissue with mature fibroblast cells figure (20).

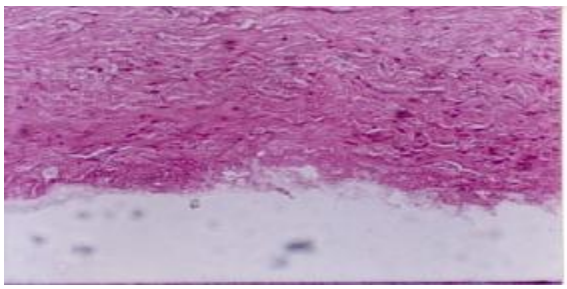

Figure 20. Polyethylene tube filled glass ionomer cement reinforced by 30% Hydroxyapatite after 10 days Hematoxylin and Eosin.X 20.

At three weeks: Thin loose fibrous tissue without inflammation figure (21).

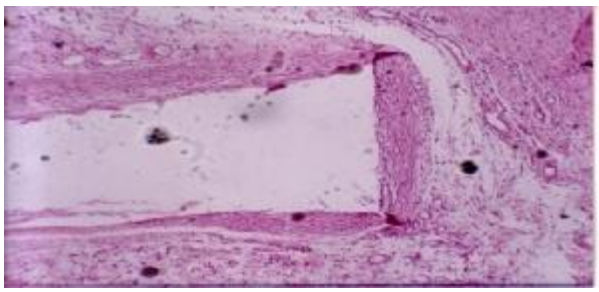

Figure 21. Polyethylene tube filled glass ionomer cement reinforced by 30% Hydroxyapatite after 3 weeks Hematoxylin and Eosin.X 20.

DISCASSION

Hydroxyaptite (HA) is the main biomineral component of human hard tissues (tooth and bone) and it's chemistry is represented by the formula ($Ca_{10}(PO_4)_6OH$) .[9,10]It is biocompatible material when synthesized artificially as biomaterial and if fluoride ions substitute hydroxyl ions on the hexagonal unit cell they give rise to fluorapatite ($Ca_{10}(PO_4)_6F_2$). [11] Associated with the biometric process, crystal growth can be used for dentistry applications on the enamel. Crystal growth conventionally involves the application of a sulphated acid solution to the enamel .[12] X-ray diffraction instruments are used to measure crystal structure, grain size, texture and/or residual stress of materials and compounds through interaction of X-ray beam with a sample.

Before introducing a new material to the market, its properties and biocompatibility must be previously studied. From a biological point of view, its irritant potential must be evaluated because eventual toxic components may cause irritation, degeneration or even necrosis of the tissues adjacent to the material. [13] This restorative cement is produced via an acid-base reaction between the glass (Ca-FAlSi) and an organic polymer acid (e.g., polyacrylic acid), which results in very interesting physical and chemical properties, such as biocompatibility, high adhesiveness compared to other restorative materials, and cariostatic properties owing to the sustained release of fluorides. [14, 15]

According to the methodology used in this study, implantation of standard polyethylene tubes containing the material for analysis, which only comes into contact with the subcutaneous connective tissue through the tubular opening on one side, as the other side is sealed, allows a comparative analysis among the experimental groups in a standardized manner, without interferences determined by variables of volume and areas of juxtaposition. In turn, the external walls of the polyethylene tube serve as control due to their low irritating potential, as a basic parameter of the ideal model of reactionary development. Thus, a comparative analysis of the experimental groups could be safely done between the test groups and between them and the control [16, 17-19]

In order to increase bonding to bone, hydroxyapatite reinforced glass ionomer cements (HA-GIC) have been developed. [20] A number of researchers have attempted to evaluate the effect of the addition of HA powders to restorative dental materials.[17] In this study, the powder of glass ionomer cement reinforced by different ratios of Hydroxyapatite (HA) has excellent biological behavior, and this is agree with other studies.[20, 21] The results of histopathological test shows that increase the percentage of hydroxyapatite may improve the biocompatibility of glass ionomer cement especially after three weeks which may related to that hydroxyapatite (HA) has excellent biological behavior, and its composition and crystal structure are similar to

the apatite in the human dental structure and skeletal system. [21]

In spite of the positive results found for the experimental GIC in this study, it is worth emphasizing that further research is needed before this material can be indicated for clinical use.

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