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Assessment of rabbit mandibular bone response to different amalgam implants radiographically

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ABSTRACT

The radiograghical interpretations can give an excellent indication about the biological response of hard tissue like bone to different foreign materials imp-lanted within it.

This clinical study was carried out to evaluate radiographically the bone reaction of four different types of Iraqi manufactured amalgam alloys (in addition to Degussa alloys as a positive control) implanted within holes prepared in the mandibular bone of the rabbit, and additional negative control group in which the hole remained empty without any implanted materials was also included.

The evaluation was done by careful verification of the presence or absence of the radiolucency at the periphery of the implanted amalgam at three different time intervals, the response varied from radio-lucency to radioopacity depending on the reaction of bone to different implanted amalgam alloys. Accordingly, the biocompatibility of the amalgam alloy was deter-mined depending on the radiographic pic-ture of bone response at the margin of the implanted alloys.

The results showed no significant difference in bone response among the different types of alloy used.

Key Words: Amalgam, bone, implant, biocompatibility.

الخلاصة

إن المعلومات الشعاعية المستحصلة من صور الأشعة يمكن أن تعطي معلومات بيولوجية قيمة عن استجابة الأنسجة الصلبة كالعظام لمختلف المواد الغريبة المزروعة فيها.

أجريت هذه الدراسة السريرية لمعرفة مدى استجابة العظم لأربعة أنواع من الأملغم السني المصنع محليا بالإضافة إلى مادة مستعملة عالميا (ديكوزا) عند زراعتها في داخل حفرة في عظم الفك السفلي للأرنب ومقارنتها مع حفرة أخرى خالية من أي أملغم.

حصل التقييم عن طريق الفحص الشعاعي الدقيق لوجود أو عدم وجود الشفافية الشعاعية على حواف الأملغم المزروع في ثلاث أوقات مختلفة بعد عملية الزراعة. وكانت الاستجابة متفاوتة من الشفافية الشعاعية إلى الظليلية الشعاعية معتمدة على مدى تفاعل العظم مع مختلف أنواع الأملغم المزروع ومدة الزراعة وحسب هذه النتيجة يمكننا معرفة مدى التطابق الحيوي للأملغم بالاعتماد على الصورة الشعاعية لتفاعل العظم على الحواف المحيطة للأملغم المزروع داخل الفك السفلي للأرنب.

بينت النتائج بأنه ليس هنالك فرق معنوي في استجابة العظم للأنواع المختلفة من حشوات الأملغم.

INTRODUCTION

Apicectomy with retrograde filling is one of the accepted surgical methods for obturating the root canal, [1] the literature supports root end filling for increased success in periapical surgery of failed root canal treatment.[2,3]

Various materials had been suggested for being used as a root end fillings but amalgam (preferably zinc–free) and re-

enforced zinc oxide eugenol cement are most commonly used. Gutta–percha, polycarboxylates, Cavit, glass ionomer cements, composite resin, zinc oxide eugenol, and mineral trioxide aggregate (MTA) have also been used with less documentation of success.[4–6]

Bhargava *et al*.[7] compare tissue reactions induced by potential retrograde filling materials. The materials used were amalgam, glass ionomer, intermediate restorative material (IRM), composite resin and gold foil. This study indicated that gold foil was the most biocompatible material followed by IRM, composite resin, amalgam and glass ionomer which showed same reaction.

One of the most popular materials used as a root ends filling was amalgam, which is composed of amalgam alloy commercially produced and marketed as small fillings, spheroid particles, or combination of these suitable for mixing with mercury to produce the dental amalgam.[8]

Two types of amalgam alloys are now in clinical use: Low copper alloy or conventional types, which contain 5% or less copper; and high copper alloy, which contain 13–30 % copper. The main difference between them is the effect that high copper content has on the amalgam reaction. The copper in these alloys is either in the form of silver–copper eutectic or Cu_3Sn form. The proper amounts of copper cause most of γ_2 phase to be eliminated within few hours after its formation, or prevents its formation entirely. Gamma 2 phase is the weakest and most corrodible phase in the amalgam and causes shorter service-ability of amalgam restoration.

The setting reaction for high copper alloy is: -

$$\underset{\gamma}{Ag_3Sn} + \underset{\text{eutectic}}{Ag–Cu} + Hg \longrightarrow \underset{\gamma_1}{Ag_{22}SnHg_{27}} + \underset{\eta}{Cu_6Sn_5} + \underset{\text{unreacted}}{Ag_3Sn}$$

So the reaction of mercury in the high copper alloy results in a final reaction with Cu_6Sn_5 (η) phase being produced rather than Sn_8Hg (γ_2) phase which is eliminated in few hours after formation due to the presence of proper amount of copper.[9]

In most of previous studies that were carried out for evaluation of biocompatibility of dental materials by implantation tests, the tested materials were implanted into the soft tissue of experimental animals like rabbits, guinea pigs, hamsters,[10,11] but in very few studies the root and filling materials were implanted into a bony tissue.[6]

In the present study, short–term implantation test was performed to assess the radiographical response of the rabbits mandibular bone induced by implantation of four types of Iraqi manufactured dental amalgam alloys, in addition to Degussa amalgam alloy, which was used as a positive control.

Currently an implant within the bone was considered successful if it exhibits no mobility, no radiographic evidence of periapical radiolucency and absence of persistent peri–implant soft tissue complication.[9]

MATERIALS AND METHODS

Materials Tested

The materials tested in this study were illustrated in Table (1). They were packed in special containers and given secret letters (A, B, C, D, E) by someone who is out of the team, so that throughout the period of the study the team could not differentiate between them (double–blind study). The proportion of alloy and mer-cury was performed according to the manufacturer instruction using dispenser then the alloy and mercury were packed in capsules which were given the secret letter of that material to be ready for use.

Experimental Animals

Eighteen New Zealand white rabbits of both sexes, 4–6 months old with aver-age weight of 1350 gm were used for this study and they numbered from one to eighteen on their backs using special paint and divided into three groups, six animals in each

group:

Group 1: The amalgam was implanted in the mandiblular bone of the rabbit and the animals were sacrificed after one week.

Group 2: Where the intra–osseous implantation of amalgam was carried out, and animals were sacrificed after four weeks.

Group 3: In this group, the amalgam was implanted intra–osseously and animals were euthanatised eight weeks post–operatively.

Table (1): Tested materials used in the study

Tested Materials	Elements Weight %			Manufacturer
	Ag	Sn	Cu	
Dentalloy (High Copper)	59%	20%	21%	Iraqi Ticonium Lab / Baghdad
Al–Rafidain Alloy	71%	27%	2%	Al–Rafidain Bureau / Mosul
Silver Alloy	70%	25%	5%	Iraqi Atomic Energy Association / Baghdad
Degussa Alloy	70%	27.2%	2.8%	Degussa Dental Co/ Germany
Dentalloy (Low Copper)	70.5%	27.2%	2.3%	Iraqi Ticonium Lab / Baghdad

Implantation Procedure

The animals were anaesthetized by intramuscular injection of mixture contain-ing 1.3 ml ketamine hydrochloride (40 mg/kg) general anaesthetic agent,[12, 13] and 0.3 ml xylazine (2mg/kg) sedative anal-gesic solution.[14] Complete anaesthesia had been obtained within 5 minutes, this dose kept the animal anaesthetized for about 40 minutes.

A small incision (about 1 cm) was made in the skin (over the submandibular area) running with the lower border of the mandible starting from the symphysis area the periosteum was reflected by blunt dis-section and mandibular bone was exposed. Three small cavities (approximately 1 cm between one and another) were drilled in the bone of each side using a slowly running round bur cooled by normal saline. The depth of each cavity was 2 mm, then each cavity was thoroughly irrigated with normal saline to remove bone particles then dried by sterile gauze.

After trituration, tested amalgam alloys were applied freshly into prepared cavities by using Messing gun. After insertion, the material was condensed to a level of bone margin and excess have been removed (Figure 1). The tested materials were implanted randomly in each animal. For the control site the same steps were performed but no materials had been implanted. After finishing the implanta-tion, the skin over the mandible was sut-ured by 3.0 black silk suture with 2 stit-ches for each.

Immediately after operation a mixture of antibiotic containing 2.5 ml procaine penicillin (500,000 IU) and 2.5 ml strepto-mycin (0.5 gm) had been administered int-ramuscularly in the

thigh muscle of the rabbits.[14] And the same dose was repe-ated every 12 hours for 3 times. During this period the animal was isolated from the remaining rabbits to avoid harming it.

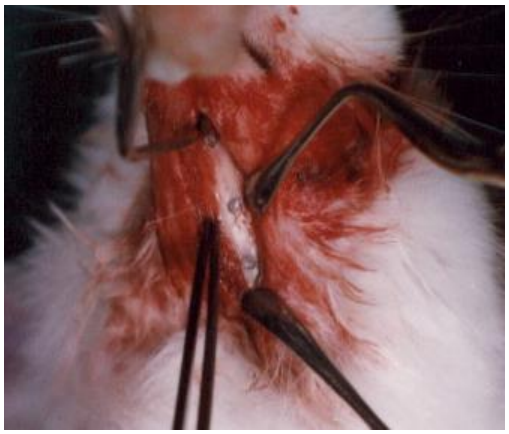

Figure (1): Amalgam condensed within the holes in the mandibular bone of the rabbit

Bony Specimens

The mandibles of the sacrificed animals were separated from the head and divided into two halves from the sym-physis, cleaned and placed in 10 % for-malin for 48 hours. After that radiograph was taken for each half separately, the film then processed and examined carefully for determinating the bone reaction to diff-erent implanted amalgam alloys and com-pare it with control group.

The evaluation was done with three examiners using viewer box and magni-fying glass in a dark room. The case was considered to be successful only when the interpretation of radiographic data was the same for all three examiners.

Reading of the radiograph was done by careful verification of the presence or absence of radiolucency at the periphery of the implanted amalgam. The case consi-dered to be negative if there is radiolu-cency at the margin of the implanted amal-gam (delayed healing), while positive when there is no marked radiolucency, or in other words there is radioopacity bet-ween the amalgam and the margin of the bone (biocompatible) and this variation depend on the reaction of bone to different amalgam implants.

The data of the radiograph were analyzed using chi–square test for the five implanted amalgam materials at three time intervals for the detection of the signifi-cance in bone formation.

RESULTS

The results of the study were recorded according to the reaction of bone to the five types of alloys and compare them to the control group at three time intervals.

Bone Reaction of Implanted alloys One Week Post–operatively (Group 1)

The bone reaction to alloys after one week was shown in Table (2) and Figure (2), which can give an excellent landmark about the biocompatibility of different all-oys.

Type A alloy showed the lowest biocompatibility (radiolucency), while the most biocompatible alloy (radioopacity) was type E when compared to control. Types B, C, and D showed an intermediate reaction.

Table (2): Bone reaction to implanted alloy after one week

Animal No.	Type of Alloy					
	CTRL	A	B	C	D	E
1	−	−	−	−	+	+
2	−	−	+	+	+	+
3	−	−	+	+	−	−

4	–	+	–	–	+	–
5	–	–	–	+	+	+
6	–	–	–	–	+	+

+: Biocompatible (radioopacity).
–: Delayed healing (radiolucent).
CTRL: Control group.
A, B, C, D, E: Represent the secret letters given to the five implanted materials.

Bone Reaction of Implanted Alloys Four Weeks Post–operatively (Group 2)

Four weeks following implantation, all the alloys showed nearly the same rea-ction which means that the biocompa-tibility was nearly equal for all the five types except type E so there was no signi-ficant difference in bone reaction among all the groups as shown in Table (3).

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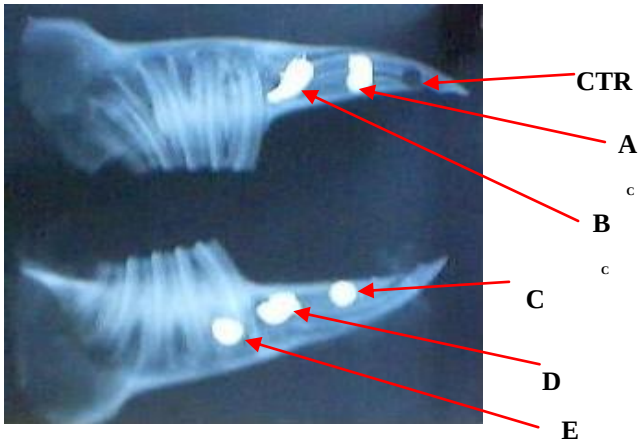

Figure (2): Radiographical image for the implanted amalgam in the mandibular bone of the rabbit after one week

(A, B, C, D, E: Represent the secret letters given to the five implanted materials; CTRL: Control group)

Table (3): Bone reaction to implanted alloy after four weeks

Animal No.	Type of Alloy					
	CTRL	A	B	C	D	E
7	+	+	–	–	–	+
8	+	+	–	+	–	+
9	–	–	+	+	+	–
10	–	–	+	–	+	–
11	–	+	+	+	+	–
12	–	+	+	+	+	+

+: Biocompatible (radioopacity); –: Delayed healing (radiolucent).
CTRL: Control group
A, B, C, D, E: Represent the secret letters given to the five implanted materials.

Bone Reaction of Implanted Alloys Eig-ht Weeks Post–operatively (Group 3)

According to Table (4) we can see that most of samples showed good rea-ction (+ve result); i.e., there is radio-

opa-city at the periphery of the implanted alloys which give an indication of good bone response to implanted materials when comparing them to control.

Table (4): Bone reaction to implanted alloy after eight weeks

Animal No.	Type of Alloy					
	CTRL	A	B	C	D	E
13	+	+	–	+	–	+
14	+	+	–	–	–	–
15	–	+	+	+	–	–
16	+	–	+	+	+	–
17	+	+	–	+	+	+
18	+	+	+	+	+	+

+: Biocompatible (radioopacity); –: Delayed healing (radiolucent).
CTRL: Control group
A, B, C, D, E: Represent the secret letters given to the five implanted materials.

The Interaction Between the Five Types at Three Time Intervals

Chi–square test was done for each group and their interactions which showed that there is only a significant difference (*p* value=0.035) at one week between the –ve and +ve subgroups (X_1 and X_2).

While there was no significant diff-erence between the remaining groups at four and eight weeks intervals as shown in Table (5) and Figure (3).

Table (5): The biocompatibility of different alloy groups with three time intervals and their interactions

Period	Total	X Group	CTRL	A	B	C	D	E	Biocom	χ^2 test	*p* value	Sig
1 week	6	X_1	6	1	2	3	1	2	–ve	12	0.035*	S
		X_2	0	5	4	3	5	4	+ve			
4 weeks	6	X_3	4	2	2	2	3	3	–ve	2.25	0.813	NS
		X_4	2	4	4	4	3	3	+ve			
8 weeks	6	X_5	1	1	1	1	3	3	–ve	4.5	0.48	NS
		X_6	5	5	5	5	3	3	+ve			

–ve=delayed healing (radiolucence); +ve=Biocompatible (radioopacity).
Biocom: Biocompatibility.
Sig: Significance; NS= Non significant; S= Significant.
X Group represent bone reaction.
X_1: –ve bone reaction; X_2: +ve bone reaction.
* Significant differences between X_1 and X_2 (p=0.035).
CRL: Control group
A, B, C, D, E: Represent the secret letters given to the five implanted materials.

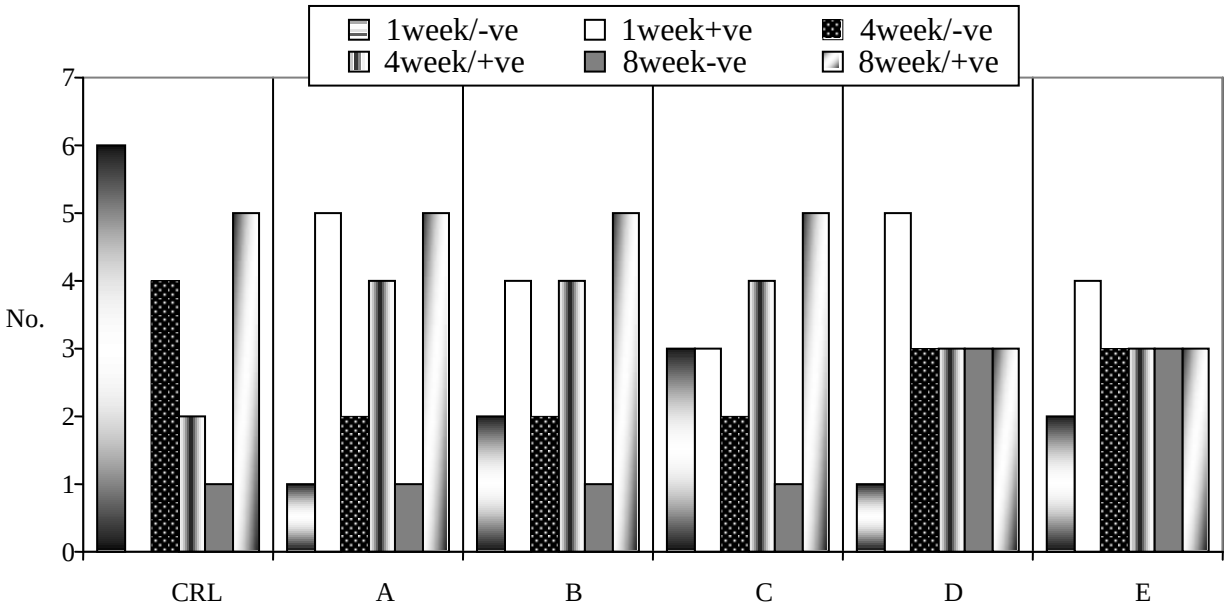

Figure (3): The bone reaction to different types of alloys at three time intervals

–ve=delayed healing (radiolucence); +ve=Biocompatible (radioopacity).
A, B, C, D, E: Represent the secret letters given to the five implanted materials.
CTRL: Control group

DISCUSSION

The results showed slow bone reac-tion to implanted alloys. This could be attributed to the fact that bone contains less amount of blood supply than soft tissue. In addition to that the implanted material was imbedded within a hole which makes it stable without any mobi-lity that cause mechanical irritation when the implanted material placed in the soft tissue.[15]

The result of one–week implantation showed that most of samples produced a negative reaction, which could be due to incomplete set of alloy during the first week due to the presence of free mercury and unreacted alloy.[16] On the other hand the implanted alloy at 4 and 8 weeks sho-wed good biocompatibility and no toxi-city. This means that the types of alloys whether conventional or high copper alloy has no significant effect on the bone reaction after complete setting, and play no role in the success or failure of retro-grade filling. So the effect was only signi-ficant after one week when the complete setting was not occur and there is unreac-ted alloy remain in the filling mass which may play roles in the bone reaction as an active ingredient. Same results obtained by Al–Nazhan *et al*.[17] who evaluated the cytotoxicity of silver amalgam and the result showed that the amalgam is non-toxic and it still the material of choice for retrograde filling.

Mattison *et al*.[16] found no significant difference between different types of am-algam up to 30 days after placement. The same result was obtained in this study which found that there was no significant difference in the bone reaction between conventional and high copper amalgam alloys.

The results of intra–osseous tissue response to the implants of amalgam in this study agreed with Austin *et al*.[18] and Yousif.[19] Those authors have also found that amalgam specimen stimulated moder-ate tissue responses at the earliest periods, then these responses decreased as time progressed indicating the biocompatibility of amalgam in bone.

CONCLUSIONS

This study found that the Iraqi manu-factured alloys showed no

difference in their bone response from Degussa when implanted within the mandibular bone of the rabbit. In addition to that the type of alloy, whether conventional or high cop-per, plays no significant role in their bone response. Accordingly, all these types of alloys can be used safely as a retrograde obturating material.

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