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Biocompatibility of Soldering Materials Used for Repair of Cobalt Chromium Joints

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الخلاصة

الأهداف: تهدف الدراسة إلى تقييم الفعاليات الحيوية لثلاثة مواد من مواد اللحام المستخدمة لربط مفاصل سبيكة الكوبالت كروميوم (وهي الحديد،الحديد الصلد، والنحاس) على أنسجة الأرنب. **المواد وطرائق العمل**: تم عمل (٩)عينات من كل من الحديد،الحديد الصلد، والنحاس على شكل اسطوانات (٣ملمX ٢,٥ملم) وكان الجزء الأوسط من مادة اللحام والجزء الأعلى و الأسفل من الأسطوانة صنعت من سبيكة الكوبالت كروميوم. تم زراعة هذه العينات في منطقة تحت الجلد في الأرنب، و بعد أسبوعين تم التضحية بالحيوان وتم عمل خزع و سلايدات والتي تم فحصها بالمجهر من قبل ثلاثة من اختصاصي أمراض الفم. **النتائج**: أظهرت النتائج أن زرعات الحديد الصلد الأكثر توافقا حيويا من بين أنواع اللحام الثلاثة، حيث إن مواقع زراعة الحديد الصلد لم تظهر أي اختلاف معنوي عن العينة الضابطة.بينما اظهر الحديد عدم توافق باختلاف معنوي عن الهينة الضابطة وفقا لأثنين من الفاحصين (A,M)، بينما لم يجد الفاحص الثالث أي اختلاف معنوي. وقد اظهر النحاس اختلافا معنويا عن العينة الضابطة وفقا للفاحصين الثلاثة. **الاستنتاجات**: اظهر الحديد الصلد الاستجابة الأكثر توافقا بين الأنواع الثلاثة من اللحام بينما الحديد من المرتبة الثانية، والنحاس، ولم تكن استجابتيهما مرغوب فيها من الناحية الحيوية.

ABSTRACT

Aims: The aim of this study is to evaluate the biological activities of three soldering materials used for joining Co-Cr joints (iron, stainless steel, and brass) on the tissue of the Rabbit. **Materials and Methods**: Nine specimens of each iron, stainless steel, and brass where made as cylinders (3mm x 2.5mm). The middle part was made of the brazing materials and the upper and lower parts were made of Co-Cr. These specimens where implanted in the subcutaneous tissue of the rabbit, after two weeks the animals where sacrificed and biopsies where made and examined under microscope by three oral pathologists. **Results:** Stainless steel implants were the most biocompatible material among the three materials used as brazing materials. Stainless steel sites had insignificant differences with the control sites. Significant difference was found between the iron implanted sites and the control group. This result found by two viewers (A, and M), while the third viewer (Z) found no significant difference between the control group, and the iron implanted sites. Significant difference was found between the brass implanted sites and the control group, this result was found by the three viewers (A, M, and Z). **Conclusions:** The stainless steel had the most favorable tissue response among the three soldering materials, iron is in the second rank, and brass showed unfavorable tissue response due to release of Cu.

Keywords: Biocompatibility, Co-Cr, Soldering Materials.

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INTRODUCTION

Cobalt Chromium alloys are commonly used to fabricate cast metal frameworks for removable partial dentures. However, clasps and connectors made of Co-Cr often fatigue and fracture from repeated insertion/withdrawal movements and force of occlusion. Broken frameworks may be repaired by connecting broken pieces, or in the case of a lost piece, by fabricating that piece with a similar or different alloy and connecting it to the framework. (1)

An alloy is a metal composed of more than one metal. Engineering alloys include the cast-irons and steels, aluminum alloys, magnesium alloys, titanium alloys, nickel alloys, zinc alloys and copper alloys. For example, brass is an alloy of copper and zinc.(2)

There are basically two types of cobalt chromium alloys; one is the Co-Cr-Mo alloy, which is usually used to cast a product and the other is the Co-Ni-Cr-Mo alloy, which is usually wrought by (hot) forging. The castable Co-Cr-Mo alloy has been used for many decades in dentistry, and in making artificial joints.[3]

Solder: to unite, bring into, or restore to a firm union. The act of uniting two pieces of metal by the proper alloy of metals.[4] The electric arc welder remains one of our most useful and timesaving pieces of shop equipment. Most of these welders are typically AC/DC, 240 volt transformer types using electricity as the energy source. Portable welders are of the diesel/gasoline engine powered type.[5] Electric Arc Welding belongs to the liquid-state category of welding processes. These involve the melting of the interface between the two work pieces to be welded. The energy supplied to produce the arc is electrical.[6]

It is fortunate that corrosion resistance can be obtained in an iron-based system simply by the addition of chromium, since, by appropriate adjustment of other alloying elements such as nickel and carbon, a wide range of microstructures can be developed. Hence, stainless steels can offer a remarkable range of mechanical properties and corrosion resistance and are produced in many grades. [7] Gray Cast Iron is probably the most common type. With slow cooling, its excess carbon solidifies as flakes of graphite. Its chief advantages are easy machinability, good damping capacity (to absorb vibrations) and relatively low cost. It is divided in further classes according to typical mechanical properties. Some types highly alloyed and with improved mechanical properties can be considered unweldable.[8] Phosphor bronze electrodes are highly desired for joining bronzes of similar composition and for welding copper, brasses and bronzes to each other, for welding them to cast iron and mild steel and also for overlying cast iron and steel.[9]

MATERIALS AND METHODS

Biocompatibility Test for the Soldering Materials: Nine samples each of iron, stainless steel, and brass soldering materials were prepared as two bars (2.5mm diameter x 3mm length) soldered with the three soldering materials with 1mm thickness. The bars were cut at 1mm distance from the soldering side. The upper and lower parts were made from cobalt chromium and the middle part is made from the soldering materials (Figure 1).

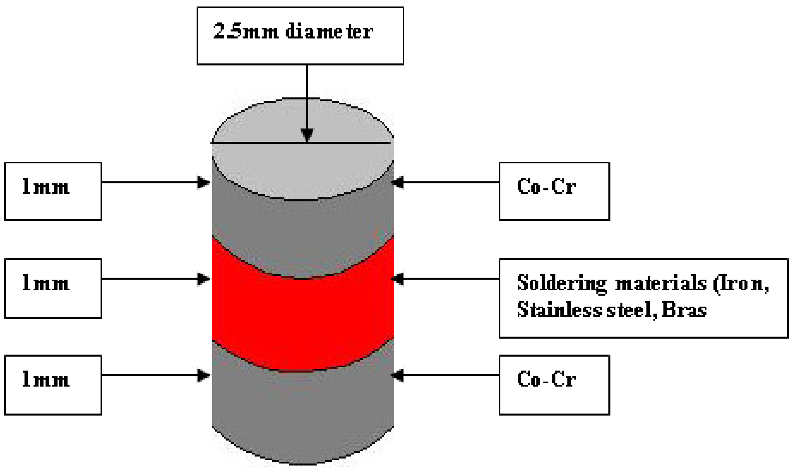

Figure (1): Biocompatibility sample

Experimental Animal: Nine local bred male rabbits, 4-6 months old with an average weight 1250-1350g weight were used. The animals were housed in an animal house specially prepared for this purpose. The animal were fed a normal diet of hay (2 times daily) and tap water.[10,11]

Description of the Experimental Procedure:

1. General Anesthesia: An intramuscular injection of a mixture containing 1.3 ml ketamine hydrochloride (40mg/kg) general anesthetic agent[12], and 0.3 ml xylazine (2mg/kg) sedative, analgesic solution.[13] Complete anesthesia had been obtained within 5 minutes, this dose kept the animal anaesthetized for about 40 minutes.

2. Implant Procedure: The anaesthetized animal was laid on its abdomen on the operation board. The fur was shaved in 3 areas of the rabbit's back on the left side of the vertebral column and another area on the right side of the vertebral column which represents the control side. A distance of about 10cm was left between one area and another. The samples were sterilized in the oven for 1 hour at 200˚C. Then the shaved areas of the skin were disinfected using 5% hibitane. Using no. 15 detachable blade on a scalpel handle, a small longitudinal incision (about 5mm) was made in the skin of each area, a pocket was created in the subcutaneous layer (by using a blunt dissection) to accommodate the implant, the sample were applied immediately in to the created pocket, the skin were sutured with one stitch of 3.0 black silk suture Figure (2).

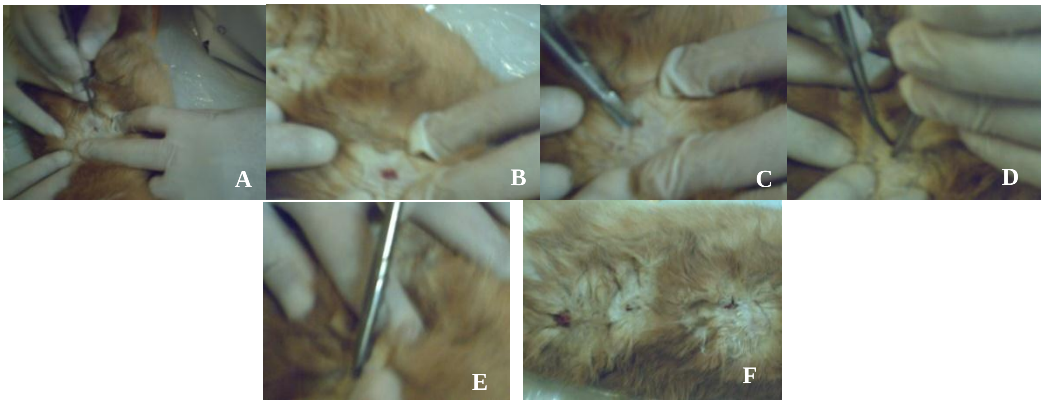

Figure (2): Surgical procedures of the implantation process

3. Post surgical Care: Immediately after the operation, a mixture of antibiotics containing 2.5ml procaine penicillin (500.000 IU) and 2.5 ml streptomycin (0.5g) had been administrated intramuscularly in the thigh muscle of the rabbit.[13] The same dose was repeated every 12 hours for three days. During this period, the animal was isolated from other animals as they will try to harm the animal or remove the suture.

4. Preparation of the Specimens for Microscopic Examination: After sacrificing the animals after 14 days, the biopsy specimen had been excised in the following manner: The sacrificed animal was laid on it's abdomen, then the implantation sites was examined grossly, the implantation and control sites were excised from the skin and kept in 10% formalin for 48 hours, processed in alcohol and xylol embedded in paraffin wax, sectioned at 5 micron thickness, stained with haematoxylin and eosin and examined under light microscope according to Luna.(14)

5. Histopathological Examination: Histopathological examination was performed according to Al-Neimee (2000) [10], who suggested a system that classify the histopathological changes that can occur during implantation of foreign materials. This system includes five grades depending upon the severity of the detrimental pathological changes associated with the implantation of various materials. The grades were as follows:

Grade 1- Only expectable inflammatory and reparative tissue responses have been seen such as infiltration of the area by an inflammatory cells, presence of oedema fluid, fibrin strands , and at late stage formation of fibrous tissue well attached to the original one, no detrimental effects have been seen. This grade was given (0)degree.

Grade 2- Mild degree of detrimental effects was observed. This grade is characterized by the presence of minimal tissue necrosis around the implanted materials and accumulation of few number of inflammatory cells such as polymorphonuclear and mononuclear cells). Some times newly formed poorly vascularized granulation tissue have been seen. This grade was given (1) degree.

Grade 3- moderate degree of tissue necrosis, presence of suppuration, fibrin strands and accumulation of large number of inflammatory cells have been seen. In addition, proliferation of fibrous tissue some times have been observed. This grade was given (2) degrees.

Grade 4- severe reaction where necrosis, inflammatory reaction around the implanted materials, and inflammatory exudate have been noticed. This grade were given (3) degree.

Grade 5- very severe reaction when an extensive amount of tissue necrosis, very large number of inflammatory cells, and extensive abscessation around the implanted material were noticed. This grade were given (4) degree. Three viewers specialist in oral pathology were chosen who had no idea about the materials implanted and had been given the criteria of the grading system, asked them to examine the slides and giving their opinion about the slides' biocompatibility each one alone. Statistical Analysis were made with Wilcoxon test at $P = 0.05$.

RESULTS

Biocompatibility Test for the Soldering Materials: Three soldering material (iron, stainless steel, and brass) were used in this study. To find the biocompatibility of these materials, they were implanted in the subcutaneous layer of the rabbit; biopsies were taken from the animal and tested by three different oral pathologists (Figure 3).

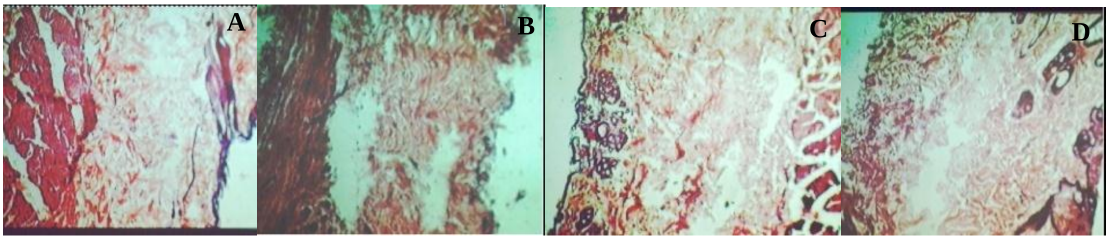

Figure (3): Biopsies taken from the implant site. A, iron material site. B, stainless steel material site. C, Brass material site. D, Control site

Biocompatibility of Iron Soldering Material: For the statistical analysis of the biocompatibility of the iron materials used in the study, Wilcoxon's test was used. Mean rank, sum of rank of the biocompatibility of iron soldering material for the three viewers were listed in Table (1).

Table (1): Mean rank, sum of rank of the biocompatibility of iron soldering material for the three viewers

Viewers	Groups	N	Mean Rank	Sum of Ranks	*P* value
A	Control	9	6.50	58.50	0.004
	Iron	9	6.50	72.50	
	Total	18			
M	Control	9	7.00	63.00	0.031
	Iron	9	10.00	88.00	
	Total	18			
Z	Control	9	5.00	45.00	0.050
	Iron	9	7.00	76.00	
	Total	18			

Where A, M, and Z; are the first letters of the first names of the three oral pathologists

Biocompatibility of stainless steel soldering material: For the statistical analysis of the stainless steel materials used in the study, Wilcoxon's test was used. Mean rank, sum of rank of the biocompatibility of stainless steel soldering material for the three viewers were listed in Table (2). No significant difference was found between the stainless steel implanted sites and the control group, this result found by the three viewers (A, M, and Z); these results were shown in Table (2).

Table (2): Mean rank, sum of rank of the biocompatibility of stainless steel soldering material for the three viewers

Viewers	Groups	N	Mean Rank	Sum of Ranks	*P* value
	Control	9	8.00	72.00	
A	**Steel**	9	11.00	99.00	0.065
	Total	18			
	Control	9	10.94	98.50	
M	**Steel**	9	8.06	72.50	0.206
	Total	18			
	Control	9	5.00	45.00	
Z	**Steel**	9	9.00	65.00	0.206
	Total	18			

Biocompatibility of brass soldering material: For the statistical analysis of the brass materials used in the study, Wilcoxon's test was used. Mean rank, sum of rank of the biocompatibility of brass soldering material for the three viewers were listed in Table (3). Significant difference was found between the brass implanted sites and the control group, this result found by the three viewers (A, M, and Z); these results were shown in Table (3).

Table (3): Mean rank, sum of rank of the biocompatibility of brass soldering material for the three viewers

Viewers	Groups	N	Mean Rank	Sum of Ranks	*P* value
A	**Control**	9	6.50	58.50	
	Brass	9	12.50	112.50	0.004
	Total	18			
M	**Control**	9	7.00	63.00	
	Brass	9	12.00	108.00	0.031
	Total	18			
Z	**Control**	9	5.00	45.00	
	Brass	9	14.00	126.00	0.000
	Total	18			

DISCUSSION

Biocompatibility of Iron Soldering Material: Biocompatibility tests of the iron materials used in this study were conducted on the basis of cell reaction of the subcutaneous tissue of the rabbit to the implanted material. Results of statistical analyses depending on the decision of three oral pathologists showed that two of them found that the tissue reaction to the iron materials was unfavorable and the material was not biocompatible, while the other reader found that no significant changes were present in the implant site (Table 2), this may be related to that in 2-dimensional cell cultures the presence of amino groups on the iron oxide nano-particles coating was mandatory for cell uptake, and this uptake did not modify cell proliferation iron oxide in biological systems, and their potential to be selectively taken up by living cells.(15) Histological analyses showed that iron oxide nano-particles did not cause long-term changes in the liver enzyme levels or induce oxidative stress and thus can be safely used for drug delivery and imaging applications.[16] However, the three viewers recorded no more than moderate cell reaction to the implanted material (iron electrode material). This tissue reaction can be explained by the conclusion of Messer and Lucas (2000) [17] who stated that it was shown that a number of cellular functions are altered in response to ions released from implant alloys. This is a first step toward developing a cytotoxicity cell culture model for evaluating the metabolic response to ions released from dental alloys.

Biocompatibility of the Stainless Steel Soldering Material: The statistical analysis results of the three viewer's readings of the implanted sites slides showed that there were no significant changes in the subcutaneous tissue of the rabbit in regard to the stainless steel materials (Table 2). These results go with the results of biocompatibility. The ability of a material to induce a "normal" response within a host is a key factor in determining the material from which to craft stents. Stainless steel, the most common inexpensive and relatively biocompatible stenting material in use nowadays has much room for improvement. [18] Stainless steel stents are considered biocompatible, because of the non-reactive oxide on the outer surface of the steel. The biocompatibility of stainless steel and titanium alloys has been documented in different cell culture systems. Results demonstrated that initial cell events such as cell attachment are unaffected by the chemical composition of the stainless steel and the titanium alloys. [19,20] This result agreed with the findings of Alvardo *et al* (2003)[21] who used austenite stainless steel.

Biocompatibility of Brass Soldering Material: The statistical analysis results of the three viewers' readings of the implanted sites slides showed that there were significant changes in the subcutaneous tissue of the rabbits in regard to brass soldering material (Table 3).There was no previous studies in this field to correlate the results with study. The presence of high percentage of copper in dental amalgam cause an extensive early irritation, [10] Zn, Cu, Hg, and Sn are metals commonly present in amalgam alloys. The continuous salivary clearance would rapidly remove these metals from the mouth, minimizing the interference with the gingival gelatinases present in saliva. However, in some cases such as amalgam tattoo and root-end filling, amalgam remains in direct contact with connective tissue for prolonged periods of time. The inhibition of gingival gelatinases activity could have a local effect on the connective tissue around this material. There are many different formulations of with physical properties that may make them behave differently in regard to gingival gelatinases inhibition.[22]

CONCLUSIONS

Under the limitation of the current investigation, this research concluded that the stainless steel had the most favorable tissue response among the three soldering materials, iron is in the second rank, and brass showed unfavorable tissue response due to release of Cu.

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