



International Journal Of
**Recent Scientific
Research**

ISSN: 0976-3031
Volume: 7(3) March -2016

REPAIR PROCESS OF THE IMMEDIATE REIMPLANTATION
OF INCISORS IN DIABETIC RATS

Alessandra Marcondes Aranega., Igor Mariotto
Beneti., Daniela Atili Brandini., Jonatas Esteves
Caldeiras.,Cristiane Mara Ruiz de Sousa Fattah.,
Tetuo Okamoto and Idiberto José Zotarelli Filho



THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
<http://www.recentscientific.com/> recentscientific@gmail.com



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 7, Issue, 3, pp. 9396-9401, March, 2016

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

REPAIR PROCESS OF THE IMMEDIATE REIMPLANTATION OF INCISORS IN DIABETIC RATS

Alessandra Marcondes Aranega³, Igor Mariotto Beneti^{1,2,3}, Daniela Atili Brandini³,
Jonatas Esteves Caldeiras⁴, Cristiane Mara Ruiz de Sousa Fattah³,
Tetuo Okamoto³ and Idiberto José Zotarelli Filho²

¹University Center North Paulista (Unorp) - São José do Rio Preto – SP, Brazil

²Education (Unipos), Street Ipiranga, 3460, São José do Rio Preto SP,
Brazil 15020-040

³Dentistry – Foa - Unesp, Araçatuba-SP, Brazil

⁴Federal University of Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article History:

Received 16th December, 2015

Received in revised form 24th

January, 2016

Accepted 23rd February, 2016

Published online 28th

March, 2016

Keywords:

Mellitus Diabetes. Tooth Avulsion.

Tooth Replantation. Periodontal

Ligament. Root Resorption.

ABSTRACT

Background: The prognostic of replanted teeth is related with integrality of cement periodontal ligament and of cement layer. This cement layer is influenced by many factors, principally the extra-alveolar period and storage local. However, there're doubts if systemic alterations like diabetes can interfere on healing process of tooth replantation too. **Objective:** The aim of this study was evaluate the healing process of immediate replantation of incisors of non-controlled diabetic rats. **Study Design:** It were used 32 rats, divided in two groups with 16 animals each group. Group I received citrate buffer intravenously. In animals of Group II was inoculated streptozotocin dissolved in citrate buffer intravenously with intention to induce diabetes. After blood glucose control the teeth were extracted and replanted immediately in their respective alveolus. Systemic antibiotic therapy started in immediate post operatory period. After 10 at 60 days of replantation, it was executed the euthanasia of animals and parts of body were submitted at laboratorial processing to obtain the cuts. These cuts were dyed with hematoxylin-eosin and trichromium of Masson for histological and histometric analysis. **Results:** Quantitative results at 60th day demonstrated that similar indexes of external root resorption and ankylosis were found in both groups. **Conclusion:** It was possible to conclude that alveolar healing process of tooth immediate replantation in diabetic rats occurred satisfactorily although connective tissue in periodontal ligament showed itself less organized in diabetic group when compared with animal of control group during all the studied periods.

Copyright © Alessandra Marcondes Aranega *et al.*, 2016, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

The replace proceeding of avulsioned teeth accidental or intentionally in them alveolus is denominated Tooth Replantation. Grater incidences of avulsion are localized in maxillary incisors and attacked more children between 7 and 10 years-old.

Its success depends on vitality maintenance of cells from cement periodontal ligament and cementoblasts (1). So, control of time that avulsioned teeth stay outside its alveolus and its maintenance in ideal storage means during this period can favor prognostic of replantation (1,2).

Some works showed that integrality of periodontal ligament becomes so affected when extra-oral period is over 90 minutes while in period over 20 minutes, the replantation success is compromised.

Pulp tissue status can influence on success of tooth replantation, because pulp can suffer necrosis and its toxins attack periodontal ligament through dentin tubules, contributing to increase of resorption process.

Farther these factors, few are known about influence of systemic alterations on healing process of replanted teeth. Among pathologies existents, there's diabetes, considering one of diseases more commons in population (3). Diabetes Mellitus

*Corresponding author: Alessandra Marcondes Aranega
Dentistry – Foa - Unesp, Araçatuba-SP, Brazil

term refer at group of metabolic disorders characterized by high level of blood glucose, from defects of secretion and/or insulin action.

Insulin has a critical role on regulation of blood glucose level (4). Its deficiency causes alterations on metabolism of carbohydrates, involving protein and lipids metabolism on the secondary form (5). Like consequence, persistence of high level of blood glucose can retard cicatrization of injury (6,7,8,9,10), alveolar healing (11) and the increase of infections predisposition (6,4,8,9,12).

Due increase of period of life, obesity and sedentariness of population, there's the increase of disease incidence (13). So, possibility of professional front a diabetic patient that suffer tooth avulsion is grater and the knowledge about it and its implications on dental treatment are indispensable. On the other hand, at this moment there is absence of studies about healing of replanted tooth on these conditions, stimulating interesting to realize this study.

This work has the aim to analyze with histological and histometric technique the immediate healing process of incisors of diabetic rats.

Study Design

After allowing of Ethic Committee on Animal Experiment (CEEAA) of São Paulo State University – UNESP / Araçatuba Dental School (annex A), were used 32 male and adult rats (*Rattus norvegicus albicans*, Wistar) with 250g of body weight.

The animals were maintained on vivarium of General Clinic and Surgery Department into common cages and were clinically free from any pathology. Before and during experimental phase, they received animal's solid food (Ração Ativada Produtor – Anderson & Clayton S.A) and free water, except during fast period (14-16 hours) that anticipate administration of streptozotocin and surgery proceeding. On this time, rats received just water. After surgery all animals were fed with triturated animal's food.

Before administration of streptozotocin the rats were divided in 02 (two) groups with 16 animals each other. Group I (Control Group) received 0,01M of citrate buffer, intravenously, pH 4,5 (Farmácia Aphoticário – Araçatuba/SP), through penile vein and in animals from Group II (Diabetic) was injected streptozotocin (Sigma-Aldrich) dissolved on citrate buffer intravenously, on concentration 35mg/kg of body weight, to induces diabetes.

On the 5th day after diabetes induction, blood was collected by rat's tail to determine blood glucose level. These levels were obtained by automatic monitoring system of glucose – Accu-Check Advantage (Roche Diagnostica Brasil Ltda).

On the 7th day, for surgery, rats received Xilazin chloride (Coopazine – Coopers Brasil Ltda - 0,03 mL/100g of body weight) and Cetamin chloride (Vetaset – Frot Dodge - 0,07 mL/100g of body weight) intramuscularly to obtain anesthetic

effect. After antiseptis on anterior maxillary region using iodine polyvinylpyrrolidone (Riodeine – Ind. Farmac. Rioquímica Ltda), right maxillary incisor of each animal was extracted according to Okamoto and Russo (14) and replanted immediately in their stock. After surgery, the animals received 20.000 U.I. (just one dose) of penicillin G-benzatine (Benzetacil – Eurofarma Laboratórios Ltda) intramuscularly.

Just 16 animals were euthanatized at 10th day and 16 were euthanatized at 60th by anesthetic overdose. Right maxillary with replanted tooth was cut on median line with scalpel blade number 15 (Embramac Exp. e Imp.) and after this, it was made a cut with right scissor on distal portion of third molar to obtain of portions. These portions were fixed in buffered formalin solution 10% (Reagentes Analíticos – Dinâmica) during 24 hours and suffered decalcification in EDTA solution 4,13%, pH 7,0. After decalcification, processing and inclusion in paraffin, it were obtained the sections with 0,6µm thickness, on root longitudinal direction. Later, a portion of each tooth was selected and colored by hematoxilín-eosin (H.E.) and Thricomic of Masson to histological and histometric analysis.

Histological analysis

Histological analysis was realized with optic microscopy, considering cervical, middle and apical thirds of lingual root face of all studied animals. It was observed some characteristics of periodontal ligament, cement, dentine, alveolar bone, further than surface inflammatory or by substitution resorption and ankylosis.

Histometric analysis

Histometric analysis used histometric sections of both groups at 60th day. It was used a digital camera (JVC TK-1270 Color Video Camera) connected at optic microscopy Carl Zeiss (Axiolab) and at computer, using Active Web Cam Program 5.1. The image of root medium part was took in two times and saved like picture (TIF24). Before, pictures were joined on Corel Photo-Paint Program (Corel Corporation 2003) with care to standardize size and position of areas in all analyzed sections.

In this study, statistical analysis considered the level of root compromising in each group by root resorption. This level was measured in pixels and represented quantity of non-resorbed dentine. The compromising was analyzed considering different types of resorption too (inflammatory and by substitution). Pictures were transferred to ImageLab 2001 Software (Diracom 3) that allowed the measures of resorbed dentine area. The quantity of non-resorbed dentine was obtained by subtraction of inflammatory resorption area and resorption by substitution from total area of analyzed dentine. Obtained data were stoked in file of Excel (Microsoft – Office) for statistical analysis.

Statistical analysis

Statistical calculum were made by SAS 8.02 Software. In this work, obtained absolute values were submitted at normality tests and Variance Analysis was selected to cross data because the sample distribution was normal.

For analysis of inflammatory resorption and resorption by substitution it was necessary a transformation of absolute values obtained after measurements in percentage and directed at scores from 1 at 4. This proceeding was necessary because in many specimens when didn't occurred the analyzed events the result was zero like representative value, becoming more difficult the application of ideal statistical test. So, score 1 represented cases of resorption absence; score 2 to cases that 0,1% at 0,5% of root surface was compromised by resorption, score 3 to cases of compromising of 51% at 99% and score 4 when 100% of root surface was compromised by resorption.

After score analysis and considering that quantitative parameters of inflammatory resorption and by substitution areas showed large variability, it was choose the use of non-parametric test Mann-Whitney for large individual comparisons.

RESULTS

It was used a qualitative analysis of two post operative periods to describe the results.

Group I – Control Group – 10th day (Figures 1 and 3)

The cement and dentine on majority of cases stay intact. However, in some points it's possible to detect the beginning of resorption of active surface, with presence of multinuclear cells. In one of specimens it's possible to observe areas with active inflammatory resorption involving cement and dentine.

Periodontal ligament show itself with vitality on cervical, medium and apical thirds on majority of cases being collagen fibers disposed on oblique and parallel form in relation to tooth vertical direction.

In some areas it's possible to observe recent bone spicules in apical third occupying the space of periodontal ligament but didn't attacked cement surface. In one of specimens immature bone tissue it's evident in contact with cement surface in a long part of medium part of root.

Group II – Diabetic rats – 10th day (Figures 2 and 4)

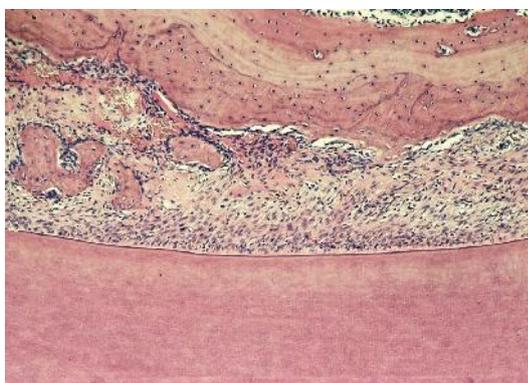


Figure 1 Group I (Control Group) – 10th Day. Medium third with preserved cement and dentine, fibroblastic proliferation, recent formation of blood vessels and collagen fibers without defined orientation. H.E. 63x.

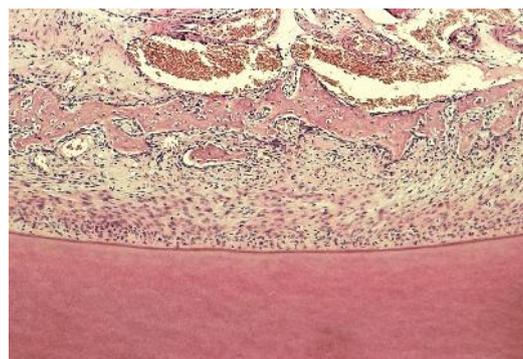


Figure 2 Group II (Diabetic rats) – 10th Day. Medium third with periodontal ligament space with fibroblastic proliferation, recent formation of blood vessels, collagen fibers without defined orientation. H.E. 63x.

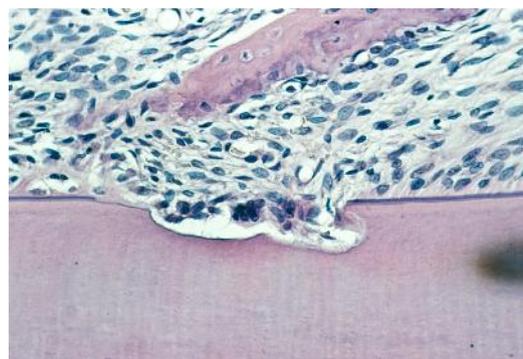


Figure 3 Group I (Control Group) – 10th Day. Area with little inflammatory resorption containing multinuclear cells, fibroblastic proliferation and recent bone formation. H.E. 250x.

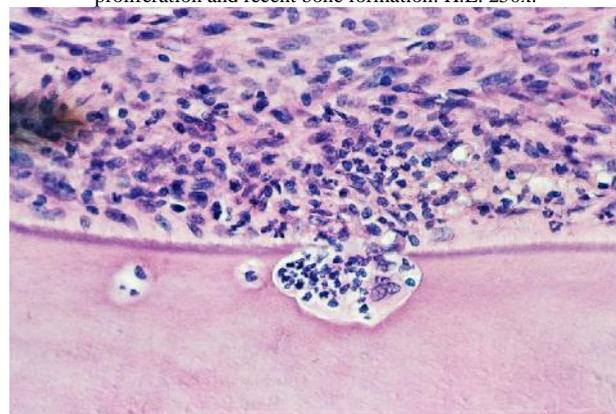


Figure 4 Group II (Diabetic rats) – 10th Day. Area with inflammatory resorption with multinuclear cells and periodontal ligament space with fibroblastic proliferation, discrete number of lymphocytes and some macrophages and polymorphonuclear neutrophils. H.E. 250x.

Cement and dentine showed integrity in near of totality, but in some points of cement surface it is evident the resorption areas being the majority of them the type of active surface resorption, whereas there're multinuclear cells. When there's involving of dentine it's possible to see some areas of active inflammatory resorption.

Periodontal ligament in two specimens is deteriorated in cervical third. In medium and apical thirds the connective tissue show moderate number of fibroblasts with discrete inflammation. Collagen fibers are disposed on oblique and parallel form in relation to tooth vertical direction. Connective tissue of diabetic animal shows recent blood vessels and is so large when they're near alveolar bone. This tissue has more

cells in this group while collagen fibers are thinner or few organized. In some points, there're recent bone spicules in area of apical part occupying periodontal ligament space but didn't attacked cement surface in majority of specimens. However, in some points of medium third there's immature bone tissue linked at cement.

Group I – Control Group – 60th day (Figures 5 and 7)

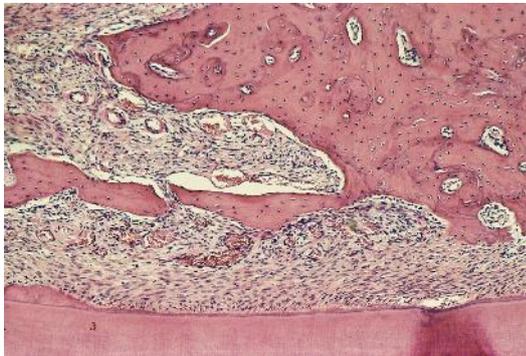


Figure 5 Group I (Control Group) – 60th Day. Medium third with preserved cement and dentine, collagen fibers with disposition on the oblique form and formation of bone tissue linked to the cement in some points. H.E. 63x.

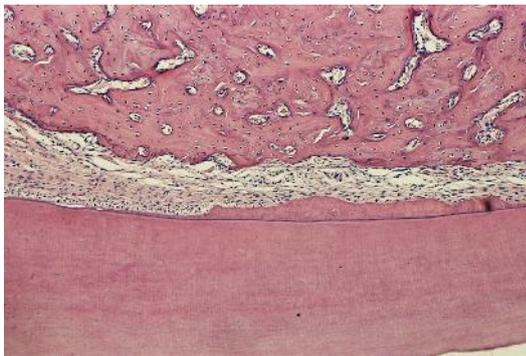


Figure 6 Group II (Diabetic rats) – 60th Day. Medium third with preserved cement and dentine, periodontal ligament space with atresia and collagen fibers without defined orientation and formation of bone tissue linked to cement in some points. H.E. 63x.



Figure 7 Group I (Control Group) – 60th Day. Area with preserved cement and dentine, periodontal ligament space with collagen fibers disposed on oblique form in relation to root. Masson's Trichromium. 160x.

Some lymphocytes can be observed in subjacent connective tissue. Some animals show bone lacunas in cervical region rounded by neutrophils with presence of active inflammatory resorption in cement and dentine. Just in one case it was observed healed area with resorption by substitution.

Cement and dentine show integrity in majority of studied specimens and for a long part of tooth surface in medium and apical thirds. Some bone formations are seen in these parts just in one of studied specimen.

Periodontal ligament is occupied by connective tissue being few developed in cervical part with presence of some lymphocytes and macrophages, but in medium part it's possible to observe a reduction of periodontal space due presence of recent bone tissue, but didn't linked tooth surface with alveolar bone. Connective tissue that stays around them is so developed showing collagen fibers disposed on the parallel form in relation to vertical tooth direction in majority of specimens.

Group II – Diabetic rats – 60th Day (Figures 6 and 8)

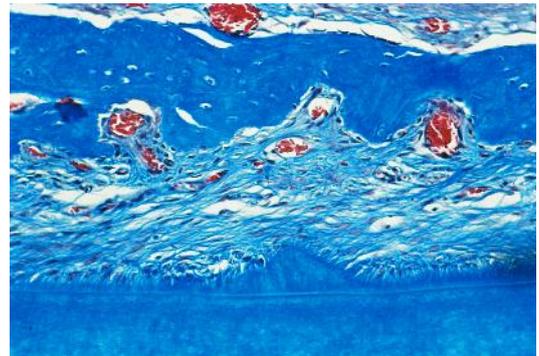


Figure 8 Group II (Diabetic rats) – 60th Day. Area with preserved cement and dentine, periodontal ligament with atresia, collagen fibers without defined orientation and space with many vessels. Masson's Trichromium. 160x.

Many quantity of resorption could be identified in this group being the majority of them localized on cervical third and active and healed resorption by substitution. Just one specimen show active inflammatory resorption on region. In relation to medium third, majority shows integrity of surface like Control Group. A higher number of bone formations could be observed on medium third especially in relation to control group.

Biggest differences among groups are observed on quality of periodontal ligament. Periodontal ligament of diabetic rats stay occupied by less developed connective tissue when compared with control group on three studied thirds of root. There's higher number of lymphocytes and macrophages especially on cervical third. On medium third there's reduction of periodontal ligament space due by presence of recent bone tissue formation on area, but didn't linked tooth surface with alveolar bone similarly at control group. There's smaller quantity of collagen fibers because there's many fibroblasts disposed on parallel form in relation to tooth surface or in some cases, without defined disposition. On apical third, connective tissue is few organized containing high number of fibroblasts.

Histometric analysis

Quantitatively, Just resorption of groups at 60th Day was evaluated. The results of histometric analysis stay in table 1.

Table 1- Number of teeth, average, standard deviation and standard wrong for comparison of non-resorbed dentine area.

	Number of teeth	Average	Standard Deviation	Significance (Group I x Group II)
Inflammatory Resorption				
Group I	10	1,3	0,48	P<0.05
Group II	7	1	0	
Resorption by substitution				
Group I	10	1,20	0,42	p>0.05
Group II	7	1,57	0,53	
Ankylosis				
Group I	10	1,20	0,42	P<0.05
Group II	7	1,29	0,49	

DISCUSSION

In this work, induction of diabetes was successful with the use of streptozocin. The diabetogenic administration in concentration of 35mg/kg by penile vein caused blood glucose levels higher than 350mg/dL in rats weighting around 250g (36) resulting clinical signals presence like polydipsia, polyuria, polyphagous and loss weight.

When cervical and apical thirds of root suffered influences resulted from forceps seizure and pulp alterations, the description of medium third for analysis of healing process of replanted teeth is more reliable in relation to cervical and apical thirds, although it's possible to describe some observations on these respective thirds.

Presence of less organized and immature connective tissue adjacent at reminiscent cement periodontal ligament indicate that diabetes interfered negatively on proliferation and function of fibroblasts and on metabolism of collagen fibers. The occurrence of these alterations in diabetic organisms was related later by other authors (5,10,11,15).

According with literature, three types of resorption can attack root surface depending on conditions of periodontal ligament and pulp tissue. Surface resorption occur especially when little parts of periodontal ligament and of cement are damaged, and so there isn't large dentine exposition and consequently, without pulp irritation through dentine canals. Inflammatory resorption normally is resultant of contaminants presence in reminiscent periodontal ligament and/or in pulp tissue. However, with loss of periodontal ligament and especially because of reminiscent embryological cells of this ligament, alveolar bone or recent trabecular bone are not blocked to link at tooth surface, causing ankylosis or resorption by substitution.

Heinthersay (16) suggested that until 30 minutes of extra-oral period the replantation should be made without endodontic treatment, because periodontal and pulp tissues are living. It's important to put avulsioned tooth on its original position as soon as possible. Similar observations can be seen in work of Andreasen (17) that related that in conditions of short time extra-alveolar, the extraction of pulp extra-oral didn't changed the extension of inflammatory resorption when compared with non-extraction.

In this work, individual analysis of resorption areas show qualitatively similar although had a discreet predominance of active inflammatory resorption and active and healed resorption by substitution in animals from diabetic group.

On this form, tooth replantation was successful on diabetic status, although in inappropriate conditions, on the contrary of later replantation (18). But in later and later times the immediate replantation can occur unsuccessful because the late of periodontal ligament organization and of bone modification could be observed on diabetic status in the same studied times. Due connective tissue was less organized, it's possible that in posterior phases can occur healing by bone tissue characterizing an ankylosis or evolution of process for a resorption by substitution. Another possibility would be the persistence of connective tissue without organization, and this could compromise functional stability of tooth. In this case, a possible contamination of connective tissue would make more serious hyperglycemic status and compromise prognostic of proceeding, contraindicating the permanence of replanted tooth.

New researches in humans could contribute to guarantee indication of immediate tooth replantation for diabetic patients.

CONCLUSION

Considering used method and obtained results in this study, it was possible to conclude that healing process of immediate tooth replantation of diabetic rats occurred on the satisfactory form although connective tissue on periodontal ligament space didn't had organization in diabetic group when compared with connective tissue of control group in both studied periods.

Competing interests

The authors declare que they have no competing interests.

References

1. Pohl Y, Fillippi A, Kirschner H. Results after replantation of avulsed permanent teeth. II. Periodontal healing and the role of physiologic storage and antiresorptive-regenerative therapy. *Dent Traumatol* 2005; 21:93-101.
2. Schwartz O, Andreasen FM, Andreasen JO. Effects of temperature, storage time and media on periodontal and pulpal healing after replantation of incisors in monkeys. *Dent Traumatol* 2002; 18:190-5.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27:1047-53.
4. Lalla RV, D' Ambrosio JA. Dental management considerations for the patient with diabetes mellitus. *J Am Dent Assoc* 1001; 132:1425-32.
5. Grandini SA. The effect of partial-pancreatectomy-induced diabetes on wound healing subsequent to tooth extraction. *Histologic study in rats. Oral Surg Oral Med Oral Pathol* 1978; 45:190-9.

6. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003 Jan; 26 Suppl 1:S5-20.
7. Rosenberg CS. Wound healing in the patient with diabetes mellitus. *Nurs Clin North Am* 1990; 25:247-61.
8. Iacopino AM. Diabetic periodontitis: possible lipid-induced defect in tissue healing through alteration of macrophage phenotype and function. *Oral Dis* 1995; 1:214-29.
9. Bailes BK. Diabetes mellitus and its chronic complications. *AORN J* 2002; 76:266-82.
10. Algenstaedt P, Schaefer C, Biermann T, Hamann A, Schwarzloh B, Greten H, et al. Microvascular alterations in diabetic mice correlate with level of hyperglycemia. *Diabetes* 2003; 52:542-9.
11. Devlin H, Garland H, Sloan P. Healing of tooth extraction sockets in experimental diabetes mellitus. *J Oral Maxillofac Surg* 1996; 54:1087-91.
12. Rayfielld EJ, Ault MJ, Kersch GT, Brothers MJ, Nechemias C, Smith H. Infection and diabetes: the case for glucose control. *Am J Med* 1982; 72:439-50.
13. Lerario AC. Diabetes mellitus tipo 2. *Diabetes News* 2004; 1:20-5.
14. Okamoto, T.; Russo, M. C. Wound healing following tooth extraction. Histochemical study in rats. *Rev. Fac. Odontol. Araçatuba*, v. 2, p. 153-169, 1973.
15. Umpierrez GE, Zlatev T, Spanheimer RG. Correction of altered collagen metabolism in diabetic animals with insulin therapy. *Matrix*. 1989 Aug;9(4):336-42.
16. Heithersay GS. Replantation of avulsed teeth. A review. *Aust Den J*. 1975Apr: 20(2):63-72.
17. Andreasen JO, Kristerson L. The effect of pulp extirpation or root canal treatment on periodontal healing after replantation of permanent incisors in monkeys. *J Endod*. 1981 Aug: 7(8):349-54.
18. Ricieri CB, Sonoda CK, Aranega AM, Panzarini SR, Poi WR, Sundefeld ML, Okamoto T. Healing process of incisor teeth of diabetic rats replanted after storage in Milk. *Dent Traumatol*. 2009 Jun; 25(3) :284-9.

How to cite this article:

Alessandra Marcondes Aranega *et al.* 2016, Repair Process of The Immediate Reimplantation of Incisors In Diabetic Rats. *Int J Recent Sci Res*. 7(3), pp. 9396-9401.

T.SSN 0976-3031



9 770976 303009 >