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ISOLAMENTO E CULTURA DE CÉLULAS TRONCO MESENQUIMAIS DA POLPA DENTAL E COMPARAÇÃO DE IMPLANTES DENTÁRIOS PARA CÃES – ESTUDOS *in vitro*

TESE DE DOUTORADO

Jaime Sardá Aramburú Junior

Santa Maria, RS, Brasil
2015

ISOLAMENTO E CULTURA DE CÉLULAS TRONCO MESENQUIMAIS DA POLPA DENTAL E COMPARAÇÃO DE IMPLANTES DENTÁRIOS PARA CÃES – ESTUDOS *in vitro*

por

Jaime Sardá Aramburú Junior

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Cirurgia Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
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Orientador: Prof. Dr. Ney Luis Pippi

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elaborada por
Jaime Sardá Aramburú Junior

como requisito parcial para obtenção do grau de
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RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Medicina Veterinária
Universidade Federal de Santa Maria, RS, Brasil

ISOLAMENTO E CULTURA DE CÉLULAS TRONCO MESENQUIMAIS DA POLPA DENTAL E COMPARAÇÃO DE IMPLANTES DENTÁRIOS PARA CÃES – ESTUDOS *in vitro*

AUTOR: JAIME SARDÁ ARAMBURÚ JUNIOR
ORIENTADOR: DR. NEY LUIS PIPPI
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Este trabalho foi desenvolvido em duas etapas, descritas no formato de artigos científicos. O objetivo do primeiro artigo foi ajustar às técnicas descritas na literatura para o isolamento e cultura da polpa dental para as condições físicas e econômicas do laboratório de pesquisa onde foi realizada a mesma. E a metodologia deste estudo foi orientada pela obtenção do tecido pulpar de cinco dentes decíduos de cães, que foram digeridos em solução simples de colagenase tipo 1 com resultados positivos para os isolamentos. O resultado do estudo foi o isolamento e cultura de células da polpa dental a um custo menor e com suficiente de células para utilização em terapia. No segundo estudo, o objetivo foi comparar a resistência *in vitro* de implantes dentários com cães de força mastigatória relatado na literatura científica. Para os materiais e métodos do estudo três diâmetros diferentes de implantes dental 3,3 mm, 4mm e 5mm foram testados quanto a resistência pela aplicação de força estática de compressão sobre eles. O resultado obtido foi de uma relação direta para a resistência entre o diâmetro e a força aplicada. Por isso, podemos concluir que o método de isolamento e cultura de células da polpa dental deste estudo é semelhante ao descrito na literatura, porém com a metodologia simplificada. E a força mastigatória dos cães pode variar, por isto é recomendado sempre que possível o uso de implante dental de maior diâmetro.

Palavras-chave: cultura celular, polpa dental, implante dental, reabilitação oral, cães.

ABSTRACT

Doctoral Thesis
Programa de Pós-Graduação em Medicina Veterinária
Universidade Federal de Santa Maria, RS, Brasil

ISOLATION AND CULTURE OF STEM CELLS MESENCHYMAL OF DENTAL PULP AND COMPARISON OF DENTAL IMPLANTS FRO DOGS – STUDIES *in vitro*

AUTHOR: JAIME SARDÁ ARAMBURÚ JUNIOR
ADVISOR: DR. NEY LUIS PIPPI
Defense Place and Date: Santa Maria, November 25, 2015.

This work was developed in two stages, described in scientific papers format. The goal of the first article was to adjust the techniques described in the literature for the isolation and culture of dental pulp cells to the physical and economic conditions of the research laboratory where the research was conducted. The methodology of this study was oriented by obtaining the pulp tissue from five deciduous teeth of dogs, which were digested in simple solution collagenase type 1 with positive results for the isolation. The outcome of the study was the isolation and cell culture of dental pulp with a lower cost and with enough cells for use in therapy. In the second study, the objective was to compare the *in vitro* resistance of dental implants with masticatory force dogs reported in the scientific literature. For materials and methods three different diameters dental implants 3.3mm, 4mm and 5mm were tested for static strength by applying compressive force on them. The result was a direct relationship to the resistance between the diameter and the applied force. Therefore, we can conclude that the method of isolation of dental pulp cells and culture of this study is similar to that described in the literature, but with the simplified methodology. And chewing strength of dogs can vary greatly, therefore we recommend possible the use of dental implants with a larger diameter.

Keywords: cell culture, dental pulp, dental implant, oral rehabilitation, dogs.

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1. INTRODUÇÃO

Os primeiros relatos sobre a odontologia veterinária nos remetem à China antiga numa época anterior Cristo, quando se realizam estimativas de idade dos equinos pelo desgaste dental. A manutenção da saúde oral era quase exclusiva desta espécie até o século XX, quando tiveram inicio as descrições de tratamento odontológico nos pequenos animais domésticos. Neste primórdio as técnicas utilizadas ainda eram simples e mimetizadas dos humanos (SAN ROMÁN, 1999). Mas atualmente, a especialidade em pequenos animais, vem num crescimento que é possível encontrar associações exclusivas em países como os Estados Unidos. Estes fatos aliados a outros, possibilitaram o desenvolvimento de pesquisassem e para animais, já que existe diferença entre humanos e cães quanto à anatomia dental, forças de oclusão e fisiologia oral (HARVEY E EMILY, 1993).

Estas pesquisas têm proporcionado o desenvolvimento de áreas específicas como a terapia celular e a implantodontia. Isto se deve, não somente a busca de melhor qualidade de vida dos animais, mas também pela necessidade de testar previamente em cobaias não humanas os protocolos terapêuticos.

Para ser possível o uso da terapia celular primeiro é necessário produzir as células tronco que teoricamente estão presentes em todos os tecidos animais (CASAGRANDE et al., 2009), isto é, podem ter origem endodérmica, mesodérmica e ectodérmica (FORTIER, 2005), pois já foram obtidas da medula óssea, pele, retina e polpa dental (GRONTHOS et al., 2002). As células tronco também são classificadas em embrionárias e adultas. O potencial de originar linhagens distintas de tecidos, a plasticidade, é o que as distinguem. E mesmo que a plasticidade das células embrionárias seja superior (BORGES E DE OLIVEIRA CALVET, 2014) as células de tecidos adultos levam vantagem pela facilidade de obtenção e por não estarem associadas a questões éticas (ISAKSON et al., 2015).

(LIN et al., 2008) relatam que os primeiros estudos com células tronco pulpar foram obtidos de cultura da polpa dental de terceiros molares humanos. E (YANG et al., 2012) isolaram e cultivaram células da papila apical de beagle. Já (DISSANAYAKA et al., 2011) produziram células de dentes pré-molares de cães. E a descoberta das células pulpares associada à biologia molecular possibilitou o

desenvolvimento de terapias orais regenerativas (CASAGRANDE et al., 2011). Para isto as culturas de células mesenquimais podem ser oriundas de dentes decíduos ou permanentes (PENG et al., 2009).

Células-tronco oriundas de cultura primária têm sido utilizadas para as mais variadas terapias regenerativas (COLLART-DUTILLEUL et al., 2015; POTDAR E JETHMALANI, 2015). Entre as aplicações terapêuticas possíveis podemos citar a regeneração óssea (YASUI et al., 2015; BASSIR et al., 2016), a regeneração periodontal (BRIGHT et al., 2015; BASSIR et al., 2016) e a busca da redução de tempo na osseointegração de implantes dentários (STEFANO et al., 2015).

Os implantes dentais apresentam formato de parafuso e são utilizados para reabilitar a cavidade oral, devido à ausência dental, pois apresentam integração com o tecido ósseo (BRANEMARK, 1983). Estes produtos até bem pouco tempo eram produzidos e comercializados somente para a reposição de dentes extraídos em humanos (HARVEY E EMILY, 1993). Mesmo que (HOLMSTROM, 1990) já tenha relatado um caso clínico reposição dentária em um cão pelo uso de implante osseointregravel. Atualmente, o uso terapêutico de implante dental em animais, pode esbarrar no custo financeiro do tratamento (TUTT, 2008).

Embora raros os trabalhos de implante dental para a medicina veterinária, os principais resultados desta técnica tem a participação de animais. O mais nobre destes resultados é a osseointegração que foi observada em tíbia de coelhos. Através da inserção de um implante de titânio que apresentava também uma irregularidade em sua superfície o que permitiu uma intima união entre tecido ósseo e superfície do metal (BRANEMARK, 1983).

A partir deste momento aconteceram estudos com animais para comprovar a integração implante/osso com grande desenvolvimento. Para estudar o assunto (BRANEMARK, 1983) utilizou implantes de titânio no formato de parafuso em cães que tiveram extraídos os dentes pré-molares e molares. Após esperar o período de cicatrização os implantes receberam as próteses. E sobre estas foram realizados testes de carga de 100 kg para a mandíbula e 50 kg para a maxila.

Ao mesmo tempo em que um houve aumento nas pesquisas que utilizam animais, faltam dados para serem empregados na terapia odontológica veterinária. Mesmo que Por isso, o uso de cobaias não humanas nos estudos com implantes

dentários seja é justificado pela similaridade dos tecidos orais de cães ecom humanos (WANCKET, 2015). Porém No entanto estes trabalhos científicos requerem algumas complementações que podem ser são obtidas através de ensaios laboratoriais *in vitro* (MATHIEU et al., 2014).

Os objetivos deste estudo é apresentar um método simplificado para o isolamento e cultura de células tronco mesenquimais obtidas da polpa dental e comparar a resistência a fadiga de três diâmetros de implantes dentários para utilizar em terapias reabilitadoras orais em cães. A revisão bibliográfica, os materiais, os métodos e os resultados obtidos estão apresentados em forma de artigo.

2. ARTIGO 1 – PUBLICADO

Journal of dentistry, Oral Disordes & Therapy, v.2, n.3, p.1-2, 2014

Isolation of stem cells from pulp deciduous teeth dog

Jaime Sardá Aramburú Junior, Tiago Luis Eilers Treichel, Saulo Tadeu Lemos Pinto Filho, Matheus Pippi da Rosa, Sergio Alexandre Gehrke e Ney Luis Pippi

Abstract

Stem Cells (SC) have the potential for self-renewal and differentiation. And some research groups used cell therapy to regenerate lost or injured tissues. With that some research groups to use in cell therapy to regenerate lost or injured tissues. The isolation of SC can be performed in various tissue origins. This paper aims to describe a protocol for isolation of stem cells from the pulp of deciduous teeth dog.

Keywords: Deciduous tooth; Deciduous tooth pulp stem cells; Dogs.

1 Laboratory of Cell Therapy, UFSM, Santa Maria, Rio Grande do Sul, Brazil

2 Cellular and Molecular Biology, Biotecnos, Santa Maria, Rio Grande do Sul, Brazil

* Corresponding author: Jaime Sarda Aramburu, Laboratory of Cell Therapy, UFSM, Santa Maria, Rio Grande do Sul, Brazil, Tel: +55-559-901-3740;

E-mail: jaimearamburujunior@gmail.com

Introduction

The Stem Cells (SC) are defined as cells capable of selfrenewal and tissue differentiation. They are present in all tissues [1,2] and can be obtained from endodermal, mesodermal and ectodermal tissues [3]. Stem cells have been isolated from: bone marrow, neural tissue, skin, retina and human dental pulp [1,4].

The Stem Cells (SC) are classified according to origin in embryonic/fetal or adult/postnatal. The difference between them is in plasticity, or the potential to produce different specialized cell lines. Since embryonic have great plasticity, but their use is still surrounded by ethical and legal issues [1].

Early studies with human SC were made with pulp tissue of extracted third molars [5] teeth. The discovery of SC dental pulp and the advancement of cellular and molecular biology led to the development of new regenerative therapies [6]. The pulp stem cells from permanent or deciduous teeth are able to provide cells for clinical application [5]. In dentistry, tissue engineering explores the SC primary or permanent teeth [7].

The possibility of using stem cells to regenerate the periodontium has motivated researchers [8] because periodontal regeneration represents a major breakthrough in periodontal therapy [9]. As Lin et al. [8], cells in bone marrow and adipose tissue regenerate alveolar bone and form a structure similar to the periodontal ligament. Tissue engineering has been aided by animal studies showing positive results [10].

Material and Methods

This study was previously submitted to the appreciation of Ethics Committee on Animal Use (CEUA) of UFSM having been approved and received the approval number: 084/2011. To this, five canines persistent deciduous teeth of dogs (Figure 1) derived from the routine of the Veterinary Teaching Hospital (HVU) UFSM were used. Each tooth after extraction was immersed in a 50 ml polypropylene tube with 10 ml of Hank’s solution (Sigma-Aldrich) at room temperature for transport to the laboratory.

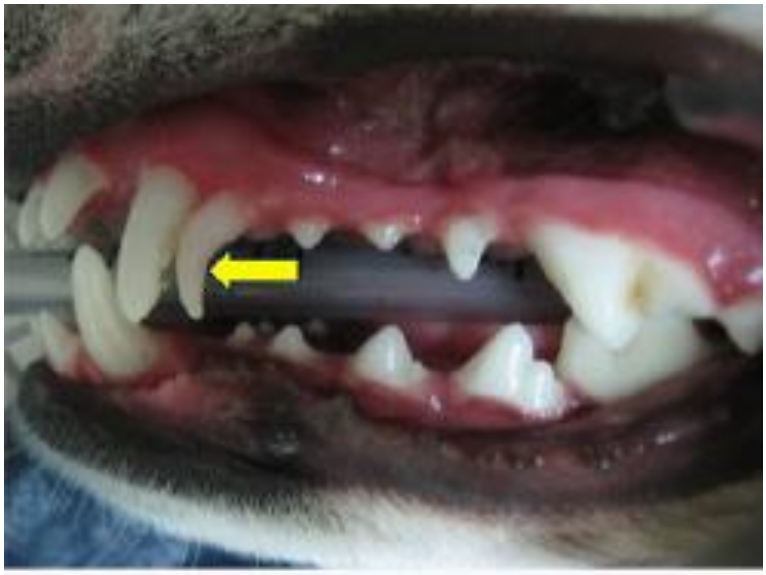

Figure 1 – Deciduous tooth from which the pulp was obtained for cell culture.

The procedure for obtaining dental pulp was carried out in the UFSM Cellular Therapy Laboratory aseptically with the materials sterilized by autoclaving and Ultra-Violet (UV) radiation. Inside the laminar flow hood, the container containing the tooth was opened and, with forceps dissection, the tooth was transferred to a Petri dish. This tooth plate was washed 3 times with Hank's solution using a 10 ml syringe. After this process, the tooth was grasped with forceps needle holder in the crown region and with the aid of a rongeur, the pulp chamber was accessed by the root apex. The Pulp Tissue (PT) was removed from the interior of the tooth with the aid of an endodontic file. Further, the PT was chopped and placed in a polypropylene tube type of solution with 0.2% collagenase type I (Sigma-Aldrich) in a water bath at 37°C for 60 minutes. The cell suspension was centrifuged at 800 g for 5 minutes at room temperature. The pellet was re suspended in DEMEM/HEPES (Gibco) culture medium, supplemented with 10% fetal bovine serum (Gibco), 100 units/ml penicillin, 100 µg/ml streptomycin (Gibco) and 3.7 mg/L HEPES (Sigma-Aldrich). Centrifuged again at 800 g for 5 minutes, while the medium was discarded and the resulting cell suspension was seeded into one well of a 6 well plate. The exchange of culture medium was after 24 hours of the initial plating and after every 2-3 days. The culture was maintained under these conditions until greater than 80% confluence (Figure 2), when it held its first passage confluence. In transplants, the cells in culture were harvested with a solution of 0.5% trypsin-EDTA (Sigma-Aldrich) and transferred to

subcultures in the respective culture medium. The subculture was maintained in monolayer until its next peal was necessary. Cultures of stem cells were transplanted up to 8 times.

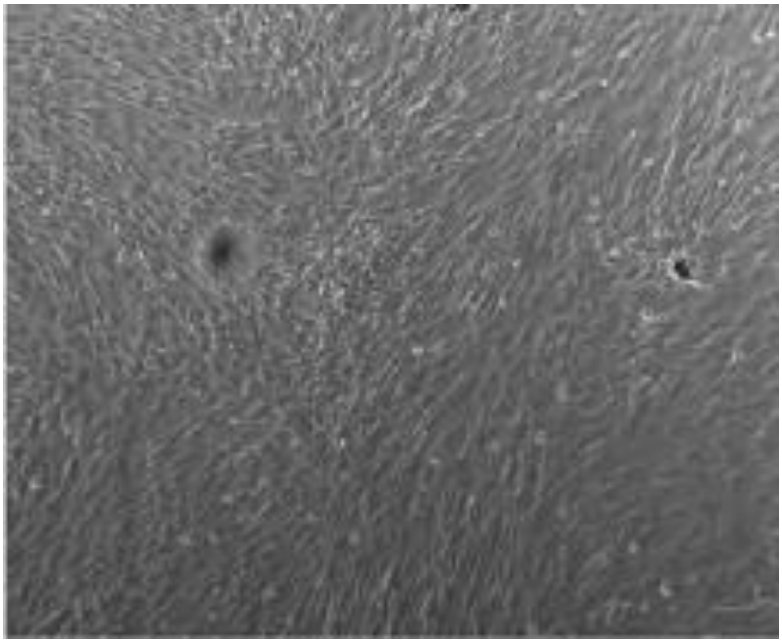

Figure 2 – More than 90% of the culture plate, suitable for performing cell confluence passage time.

Results and Discussion

Isolation and culture of pulp tissue was considered positive, since 80% of the samples showed cell growth after 24 hours of cultivation. Luisi et al. [11] observed a similar result.

The methodology for storage and transport of biological material from the time it was collected until processing differs from Bernardi et al. [12], but proved to be efficient regarding contamination, since none of the cell cultures was affected. It is believed that the prior training of the team was determinant to the result.

Access to dental pulp was safe and effective only with the use of alveolotomo while Bernardi [13] made use of high rotation and drills, which is not always present in lab equipment. Unlike Miura et al. [14] who added to this dispase solution. With this, protocol becomes more practical, less expensive and is also financially efficient for cell proliferation.

In considering the description of Peng et al. [8] about the easy and rapid expansion of stem cells from the dental pulp of human deciduous tooth in vitro, we can mention that the dental pulp of deciduous teeth of dogs had similar behavior, because it got the proper confluence mobile ringing in the shortest time quoted in the literature [11].

Meeting isolation and cell culture are requirements to work with regeneration of tissue when using cell therapy [8]. The protocol suggested in this paper can help new groups that have an interest in this research line.

Conclusion

The protocol suggested by our group is efficient for the isolation of dental pulp of deciduous teeth of dogs and features a lower financial cost if the methodology used by other research groups to obtain stem cells were purchased.

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3. ARTIGO 2 – A SER ENVIADO PARA PUBLICAÇÃO

Dental implants in veterinary dentistry: mechanical test and clinical considerations

J.S. Aramburú Junior [a], N. L. Pippi [a], J.A. Shibli [b], S.A. Gehrke [b,c, *]

[a] Post-Graduate Program in Veterinary Medicine, Federal University of Santa Maria, Santa Maria, Brazil.
[b] Department of Periodontology and Oral Implantology, Dental Research Division, University of Guarulhos, Guarulhos, SP, Brazil.
[c] Director of Biotecnos Research Center, Santa Maria, Brazil; Professor of the Catholic University of Uruguay, Montevideo, Uruguay.

Abstract

Recently, dental implants began to be used for the rehabilitation of lost teeth in veterinary dentistry. However, some observations should be considered during use, such as the resistance of these parts. Then, the aim of the present *in vitro* study was to assess resistance to static fatigue of dental implants. Sixty implants and abutments were used with the smallest diameter of each model. Three groups (n = 20) were created on the basis of the implant diameter, all with external hexagon: Ø3.30 mm (group 1), Ø4.0 mm (group 2) and Ø5.0 mm (group 3). All groups were subjected to quasi-static loading at 30° to the implant axis in a universal machine. The mean fracture strengths for group 1 was 964 ± 187 N, for group 2 was 1618 ± 149 N and for group 3 was 2595 ± 161 N. Significant differences between the groups with respect to resistance after the load applications was observed ($p<0.05$). The diameter of implants affects the resistance to external forces during the application of non-axial strength and must be observed during the planning of rehabilitation to avoid problems.

Keywords: veterinary dentistry, animal rehabilitation, dental implants, fracture mode, fracture strength.

Introduction

The advance of the dental implant within the human afforded the possibility of rehabilitating patients with different types of tooth loss (single or multiple) and thereby improve their quality of life. Currently, the use of dental implants in the dental clinics is a reality, because there was a great evolution of knowledge and improvement of techniques generating a great reliability in this technique. Long term human studies report a success rate of over 90% (Friberg *et al.*, 2008). In addition, most of the advances and knowledge acquired in this specialty were obtained in animal studies (ie, monkeys, dogs, sheep, pigs, rabbits and rats, mainly) (Natiella, 1988; Pearce *et al.*, 2007; Stadlinger *et al.*, 2012; Vignoletti e Abrahamsson, 2012).

On the other hand, with a much lesser extent, animal dentistry is gaining ground because the increase in animal care in day-to-day lives is a reality. Thus, the care of the oral health who until recently was an exclusively human beings, has become a routine also for animals. However, many times, tooth loss due to infection, injury or malformation affect animals and can hinder your chewing function and sometimes aesthetic appearance (Williams, 1986). So, why not replace those lost dental pieces with dental implants, since it is such a reliable technique.

Tannenbaum *et al.*, 2013 published in his article the factors that could contraindicate the use of dental implants in dogs and cats, such as the lack of proper hygiene, surgical risks, costs, within others. In addition to these problems properly reported, a major problem that must be considered is the difficulty in determining premature contacts installed in rehabilitation and masticatory strength of these patients, which may vary widely, Ellis *et al.*, 2008; Ellis *et al.*, 2009 as over-charge can result in the loss of osseointegration of the implant or even its fracture implant depending on the model used. Then, the objective of this study was to evaluate *in vitro* resistance of dental implants made of titanium and make a correlation with the masticatory force data reported in the literature for small animals.

Materials and methods

Sixty dental implants and 60 abutments manufactured in titanium commercially pure (CP), with external hex connection. Three implant diameters were used in the final analysis: Ø3.30 mm (group 1), Ø4.0 mm (group 2) and Ø5.0 mm (group 3). Fig. 1 illustrates the dimensions and appearance of the implants used in this study (13 mm in length).

Figure 1: Images of the implants used in this study.

Test implants were loaded with static compressive forces. The static fatigue strength of the dental implants was tested according to previous guidelines; these guidelines recommend embedded in epoxy resin with the cervical part of implant body placed at 3 mm above surface level, simulating various marginal bone levels. The epoxy resin had a Young's modulus of elasticity similar to cortical bone. Furthermore, the implants were positioned with an angulation of 30° with respect to the applied load (Fig. 2) (Standardization, 2007). For each group, twenty sets (implant/abutment) were used.

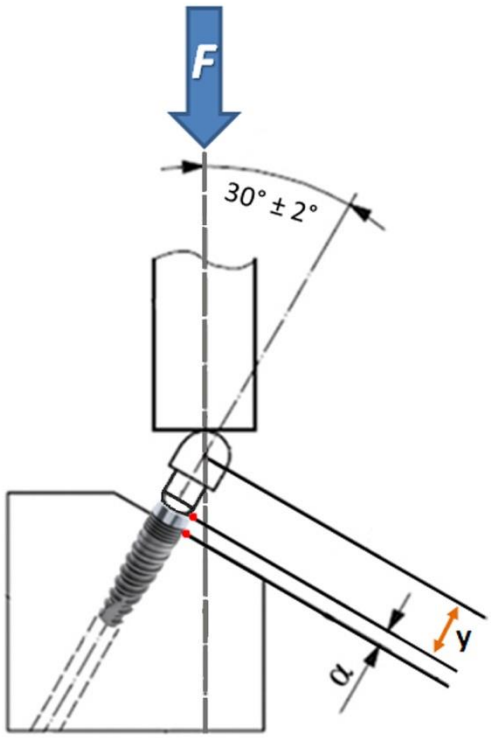

Figure 2: Scheme used in the compression test, based on ISO 14801/2007 standards [6]. The distance α was 3 mm, corresponding to the implant without insertion. The distance *y* was respected in all samples (*y* = 10 mm).

The abutments were screwed in the implants, with the same final height, and received a torque of 32 N, as recommended by the product manufacturer. A metal hemisphere was elaborated and cemented on the abutment to simulate a dental crown. All samples were subjected to quasi-static loading until fracture using a properly calibrated universal testing machine (model AME-5kN, Técnica Industrial Oswaldo Filizola Ltda, Guarulhos, Brazil) with a test capacity of 5.0 kN. The test speed was set at 1 mm/min, similar to previous studies conducted by our team (Rosnow *et al.*, 2000; Gehrke *et al.*, 2014; Gehrke, 2015). Tests were conducted at the Testing Laboratory of Biomechanics (Biotecnos, Santa Maria, Brazil).

After the quasi-static loading test, all fractured samples were ultrasonically cleaned in 96% isopropanol and observed under low-power magnification. The images were taken using a digital stereomicroscopy of the surface (Cnoec, Opto-Edu, Beijing, China) in two magnifications (10 and 100 times), and the data were reported descriptively.

Statistical analyses were performed using a one-way analysis of variance (ANOVA) to determine the differences between the three groups (α=0.05 for significance). To interpret the effect sizes of the samples was used the Cohen's a method.[14]

Results

The fracture strength values of all groups recorded during quasi-static loading are shown in box plots graph of the Fig. 3.

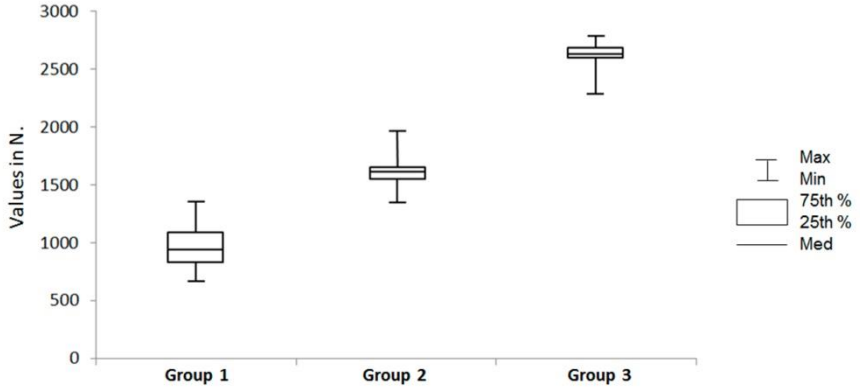

Figure 3: Box plots graph of the implant resistance in the three groups.

The external hex implants (Group 1) showed a slight deformation to the hexagon and in the lateral basis of the platform of the implants was observed, with an average strength of 964 ± 187 N (Fig. 4). In the Group 2, the samples showed a severe deformation in the hexagon and in the lateral basis of the platform of the implants was observed, showing an average of 1618 ± 149 N (Fig. 5). While, the Group 3, as expected showed the highest values of resistance, with an average strength of 2595 ± 161 N. There was a deformation in the hexagon and in the lateral basis of the platform of the implants (Fig. 6). No tear (fissure) of the implant body was detected.

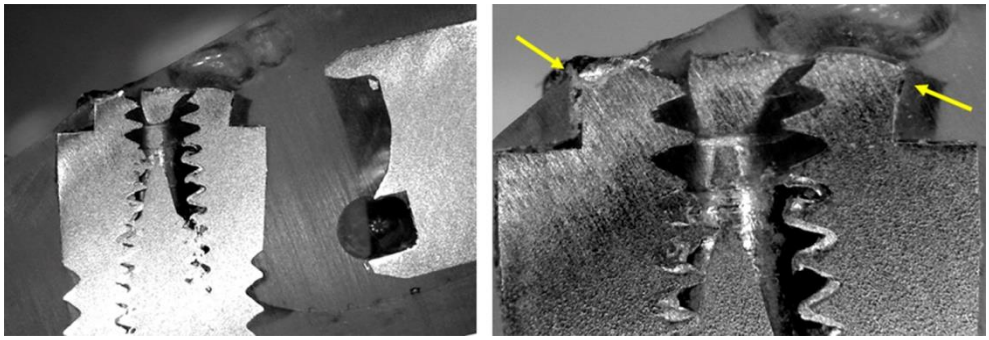

Figure 4: Image of the sample of group 1, where in the greater increase is possible observer damage hexagon of the implant (yellow arrows).

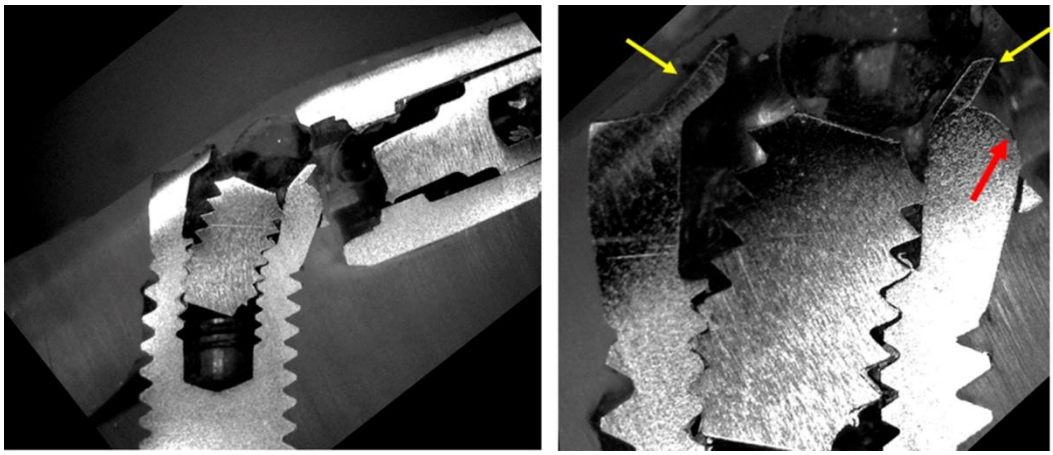

Figure 5: Image of the sample of group 2, where in the biggest increase is possible observer damage hexagon of the implant (yellow arrows) and in the platform base (red arrow).

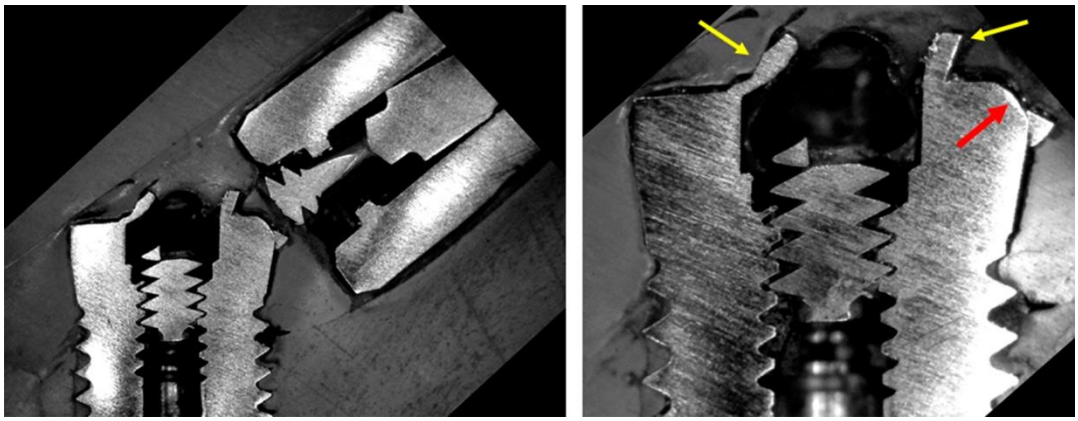

Figure 6: Image of the sample of group 3, where in the biggest increase is possible observer damage hexagon of the implant (yellow arrows) and in the platform base (red arrow).

Significant differences between the three groups were observed using a one-way ANOVA test, where F-cal = 486.1006 was greater than F-crit = 3.1588, with significance set at $p=1.54 \times 10^{-36}$.

Discussion

Endosseous implants are widely used for prosthetic treatment in fully or partially edentulous in humans patients. In general, these implants are considered consistent and predictable, with few failures (Pylant *et al.*, 1992). In situations where implant fracture occurs, it is difficult to repair the implant because of technical and physiological complications. The possible causes of fracture can be classified into three broad groups: 1) failure of the implant design or the employed material; 2) an absence of passive adaptation of the prosthetic crown to the implant substructure; and 3) overload due to parafunctional habits. The type of treatment may also be influenced by the load and stress that is transmitted to the implant following reconstruction. The results of this study demonstrated that the implant diameter can significantly influence the level of resistance offered by the implant to external forces.

Some authors, (Tagger Green *et al.*, 2002) have observed that bone loss occurs around the implant above its point of fracture, particularly when molar implant units are involved. Corono-apical resorption produces a high bending stress on the implant because of the loss of bony support. Bone resorption in response to peri-implantitis usually extends to the level of bone corresponding with the end of the abutment screw, where resistance to bending is diminished (Rangert *et al.*, 1995; Sanchez-Perez *et al.*, 2010). This region is strongly related to the magnitude and direction of the stress that is transmitted to the implant. These forces are affected by the nature of the antagonist teeth, the bite force, the number of implants available to support the load and the structure of the prosthesis with respect to the position of the implant (Albrektsson *et al.*, 1986). Here, we examined the resistance to static fatigue of implants with different diameters and found significant differences between the models. This study was conducted in accordance to ISO 14801:2007 with the set (implant/abutment) at an inclination of 30°, (Standardization, 2007) because theoretically it would be the most critical inclination for the implants in function.

The estimated biting forces in domestic dogs was 147 at 3417 N in comparison of post-mortem and in vivo measurements, (Ellis *et al.*, 2008) while in another study the measured values of the biting forces were between 375 at 1606 N

(Ellis *et al.*, 2009). The variation range of the values presented by the authors above is quite large, staying much higher than the maximum average resistance found for the implants pf the group 1 (964 ± 187 N) and the group 2 (1618 ± 149 N). Only the large diameter implants of the group 3 (2595 ± 161 N) were closest to the maximum values for the strength of animal bite. However, the diameter of the implant is dependent on the thickness of the underlying support bone, which is critical ratio for successful treatment.

The fracture load values found for the titanium implants in this study are lower than those reported by (Strub e Gerds, 2003) for implant-metal abutment combinations after chewing simulation. This difference may be explained by differences in methodology. For example, in this study the static load measurements were stopped after a deflection of 4 mm, while (Strub e Gerds, 2003) continued until they observed a deviation from the linear slope in the load displacement graph. The fatigue test established by ISO 14801:2007 (Standardization, 2007) is extremely important in the evaluation of dental implants. These guidelines serve to analyze the samples mechanically with the intention of mimicking clinical behavior. Our tests using static implant fatigue for different diameters demonstrated that implant strength is critical in implants with small diameters. These results demonstrate that the diameter of implant and abutment can change the performance and resistance of the system, and suggest that in areas where there is a possibility to use implants with highest diameter, is an important consideration to the longevity of the implant system in dental repair. While other meaningful results have been reported in chewing simulation or fatigue loading studies of implant abutment systems, (Cibirka *et al.*, 2001; Gratton *et al.*, 2001; Khraisat *et al.*, 2004) clinical trials are necessary to validate the results of these investigations as well as the present *in vitro* study.

Conclusions

Within the limitations of this study, we concluded after testing *in vitro* of external forces that resistance of the implants is affects the diameter , so we recommend the use of dental implant five millimeters diameter, for oral rehabilitation in dogs.

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4. DISCUSSÃO

Os estudos com células tronco permitem que haja um melhor discernimento sobre a regeneração tecidual. O primeiro passo da terapia é a escolha da fonte tecidual para o estabelecimento da cultura. A medula óssea até bem pouco tempo era o tecido mais utilizado para a obtenção de células, no entanto o processo de coleta pode ser muito lesivo ao doador. Por isto tem se utilizado tecidos considerados de descarte, por exemplo os dentes terceiros molares como fonte de obtenção da polpa dental (DERAKHSHANI et al., 2015). Em nosso estudo foi possível isolar e cultivar o tecido pulpar de dentes decíduos, ou dentes de leite, de cães. Este material também é considerado de descarte, pois fisiologicamente sofrerá a exfoliação para o surgimento do dente permanente. Células tronco da polpa de dentes decíduos também foram obtidas por Kashyap (2015). Conforme já haviam relatado Miura et al. (2003) em seu estudo a obtenção de células tronco do tecido pulpar de dentes decíduos.

Para o isolamento das células tronco o tecido pulpar após ser removido do dente deve ser imerso numa solução com enzimas digestivas. No estudo conduzido por (RICCIO et al., 2010) foram utilizadas uma mistura de colagenase tipo I e dispase por um período de uma hora numa temperatura de 37°C. Corroboram Gronthos et al. (2000) que obtiveram uma cultura de células da polpa dental com uma solução bem semelhante. No entanto nossos resultados demonstram ser possível isolar celular do tecido pulpar a partir de uma solução simples de colagenase tipo I. O mesmo protocolo utilizado pelos pesquisadores (BANSAL E JAIN, 2015) que também isolaram células da polpa dental.

Após a extração das células do tecido pulpar, a cultura é mantida numa solução de Meio Eagle Modificado por Dulbecco (DMEM), suplementada com 10% de soro fetal bovino e antibióticos e incubada em um ambiente a 5% de CO_2 até o momento de sua utilização conforme descreveram Conde et al. (2015). Protocolo similar ao descrito anteriormente por Ferro et al. (2012) e ao protocolo seguido em por nosso grupo de pesquisa para a realização deste estudo.

Células tronco isoladas e cultivadas da polpa dental conforme descrito em nossa pesquisa podem ser utilizadas para estimular o reparo ósseo (WANG et al.,

2013), prevenir a perda do ligamento periodontal e promover a regeneração do complexo dentina-polpa (FENG E LENGNER, 2013), induzir a regeneração dos tecidos pulpares (DEMARCO et al., 2011) e através da bioengenharia ser possível a restauração de tecidos lesados (LYMPERI et al., 2013).

Ao estudarem *in vivo* a força mastigatória de cães Brunski e Hipp (1984b) consideraram que esta pode influenciar no sucesso biomecânico do implante dental. No entanto poucos são os trabalhos científicos que nos permitem avaliar a força de mastigação de cães. ELLIS et al. (2008 e 2009) estimaram *in vivo* e em *ex vivo* uma força que pode varia de 147 até 3417 N. Após esta observação ficamos incentivado para estudarmos *in vitro* a ação da força mastigatória em três diâmetros de implantes, uma vez que as pesquisas de implantes dentários com animais relatam apenas o comportamento da osseointegração e dos tecidos periimplantares (WANCKET, 2015).

Tannenbaum et al., (2013) descreveram que a força mastigatória é um fator que contradiz a indicação de uso de implantes em cães, pois pode variar muito a cada paciente. No trabalho de Brunski e Hipp, (1984b) eles relataram que a consistência da dieta fornecida aos cães também afeta o valor da força de mordida. Esta variabilidade de fatores fisiológicos que os cães apresentam pode ser superada pela escolha do diâmetro adequado do implante dental conforme foi demonstrado em nossa pesquisa.

A força máxima de mordida de um cão pode superar a de humano em até três vezes ao compararmos os estudos de (BRUNSKI e HIPP, 1984a) e (ELLIS et al., 2008). Ao levarmos em conta que a resistência do implante dentário pode ser afetada pelo seu diâmetro, nossos resultados demonstraram que os implantes de 5 mm, suportaram uma força de carga. Ao compararmos com os dados da literatura encontramos um resultado semelhante à máxima registrada no estudo de Ellis et al. (2008) e duas vezes superior à relatada por Brunski e Hipp (1984a). O que nos permite sugerir o uso de implantes dentários de 5 mm de diâmetro como o de maior resistência as forças mastigatórias em cães.

Kim et al. (2009) utilizaram implantes de 3,75 mm de diâmetro em cães para analisar a estabilidade durante oito semanas, descobriram que clinicamente a estabilidade do sistema é superior em áreas de maior contato ósseo. O que é

corroborado por (TAGGER GREEN et al., 2002), pois para estes autores, a perda óssea periimplantar pode facilitar a fratura do implante. Com isto, sugerimos que a escolha do implante deverá ser sempre para o de maior diâmetro entretanto devemos observar a quantidade óssea da área e os hábitos parafuncionais do cão.

5. CONCLUSÃO

Este estudo nos permite afirmar que a metodologia utilizada para a obtenção das células tronco mesenquimais provenientes da polpa dentária de cães é mais simples e menos onerosa que outros protocolos citados na literatura científica para terapia celular. Em relação ao estudo sobre implantodontia, ao associarmos a variação na força mastigatória dos cães aos testes de resistência *in vitro* de três diâmetros de implantes dental, nos faz sugerir o uso de implante de 5mm de diâmetro para a reabilitação oral de cães.

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