THESIS SUMMARY.

Histochemical variation in the processes of rhizolysis in primary dentition teeth.

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INTRODUCTION

During tooth development, many factors contribute to the growth and to the differentiation of cells associated with the formation of hard and soft tissues. In the dental pulp, it has been shown that the interaction between epithelium and mesenchyme is critical for dental organogenesis. This interaction is regulated in the extracellular matrix (ECM) *(Figure 1)*, by molecules that mediate an ordered and specific sequence of events.

The ECM is composed of *glicosaminoaglicans* (hyaluronic acid, chondroitin-4 sulfate, chondroitin-6 sulfate, dermatan sulfate, heparan sulfate and keratan sulfate) and *adhesive glycoproteins.*

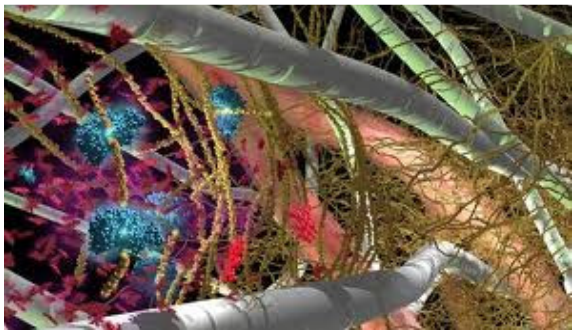


Figure 1. Scheme of the extracellular matrix
Source: www.biologiamedica.blogspot.com
(Consulted on 03/26/2012)

With the exception of hyaluronic acid, *glicosaminoaglicans* presents sulphate radicals therefore they present a dominant negative charge; this situation attracts a cloud of cations (especially sodium) which is osmotically active, recalling water, which explains the high hydrophilicity of these compounds and the formation of a gel in the ECM. This quality provides to the connective tissue the ability to resist compression forces. At the same time, hyaluronic acid provides elasticity and lubrication to many types of connective tissue, especially noticeable in the case of articular cartilage. It facilitates cell motility and interaction with collagen. It is known that the acid groups of these compounds interact with collagen basic radicals, contributing to the structure (firmness) of the ECM.

Adhesion glycoproteins can bind to specific receptors of the plasma membrane, establishing continuity between the interior and the extracellular space of the cell. The reason for the adhesion molecules denomination is because, for a molecule to be able to act with another of adjacent cell surface or of the surrounding ECM, both must established contact by the formation of intermolecular bridges. The essential purpose of the interaction between two molecules is the information passing from one cell to another. We distinguish the cell

adhesion molecules (immunoglobulins, cadherins and selectins) and the substrate adhesion ones (integrin, fibronectin, laminin, metalloproteinase and CD44, among the most important). *(Geneser Finn, 2000; Adams, 2001; Thomas and Speight, 2001, Butler, Brunn and Qin, 2003; Maillet, 2006; Abraham, 2006; Pizzi and Crowe, 2007; Bertl et al, 2009; Schmidt and Friedl, 2010)*

The CD44 molecule is one of the most important substrate adhesion molecules. It is a transmembrane glycoprotein involved in the adhesion between the cell and the different components of the ECM. It belongs to the superfamily link to hyaluronate or hyaladherinas binding proteins. The connection between hyaluronate receptor and CD44 was initially suggested in 1989 when it was reported that the extracellular domain of CD44 is homologous in the hyaluronic binding region to the link protein of the cartilage, suggesting that CD44 may be to bind hyaluronate and thus closely resembles the receptor. *(Lesley et al, 2000)*

The distribution of CD44 in the human tooth germ corresponds to that of hyaluronate in most locations, suggesting that during tooth development this transmembrane protein plays an important part in hyaluronan-mediated events.

In the processes of rhizolysis, the CD44 molecule is adhere to actin microfilaments present on odontoclasts border *(Figure 2)*. Indeed, the resorptive process begins when the odontoclast adheres to the bone matrix. The most surprising and unique characteristic of the cytoskeleton of the odontoclasts is at sites of cell-substrate adhesion.

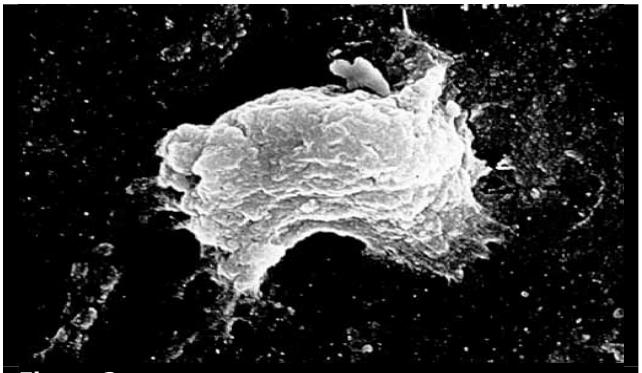


Figure 2.
Odontoclasts Image (Scanning electron microscopy x 4500).
Source:
http://www.conganat.org/iicongreso/conf/018/osteocl.htm
(consulted on 03/26/2012)

The odontoclasts organize its cytoplasmic elements polarizing areas of the basement membrane that allows the Golgi apparatus the excretion of lysosomal enzymes that are not release to the ECM to be trap against the bone, where the resorptive process will happen. There, with the bone surface and below the ruffled of odontoclasts cell surface as borders, a sealed spacer is created with an acidic microenvironment in which the resorption process will happen.

Interaction of CD44-hyaluronic acid causes a rapid activation of surface protein degradation (proteolysis) by family metalloproteinases (known proteins that act in dental rhyzolisis process); these proteins breakdown the CD44's external domain molecules and thus limit cell adhesion.

Applying these molecular concepts and considering that the physiological events that are involved in dental replacement processes generate histochemical changes that induce root resorption process, it was exposed the hypothesis that the magnitude of the presence of CD44 in the dental pulp is in correlation with the complexity of root resorption. To corroborate this, the objective of this study was to determine the concentration levels of CD44 through a specific marker, hyaluronic acid, in different stages of root development and in inflamed pulp tissue involutional stages, healthy and supernumerary of primary dentition teeth.

MATERIALS AND METHODS

Patients

We worked with patients treated at a private dental practice and those treated in the FOR-UNR Pediatric Dental Care.

As an inclusion criteria there were selected pieces that, for various pathologies, different dental treatments in deciduous teeth that involved pulp extirpation or extractions; supernumerary teeth were also included. Patients with systemic disease that may influence the biological repair response were excluded.

Sample

The pulp tissue was from 10 deciduous teeth derived to endodontic with a diagnosis of irreversible pulpitis and from 12 deciduous teeth with healthy pulp which for orthodontic reasons extractions must be performed; pulp tissue from 6 supernumerary teeth that had to be extracted because alterations in dental arch location were also included.

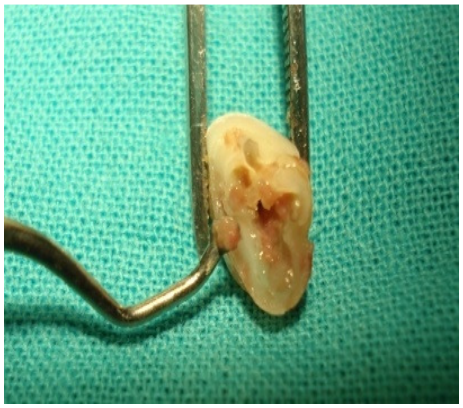


Figure 3. Dental pulp removal

For each tooth included in the sample a data collection form was completed that included a formal consent, considering the ethical standards of the UNR.

For pulp removal of the teeth it was made the opening of the pulp chamber and with Black type spoon the pulp was extracted *(Fig. 3)*. All pulp samples obtained were immersed in saline in Eppendorf tubes and frozen at -20 ° C until processed.

CD44 dosage technical description

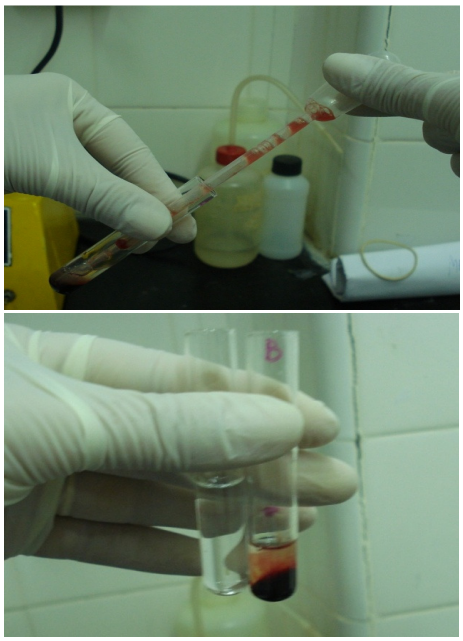


Figure 4. Washing and suspension of red cell in phosphate buffered saline

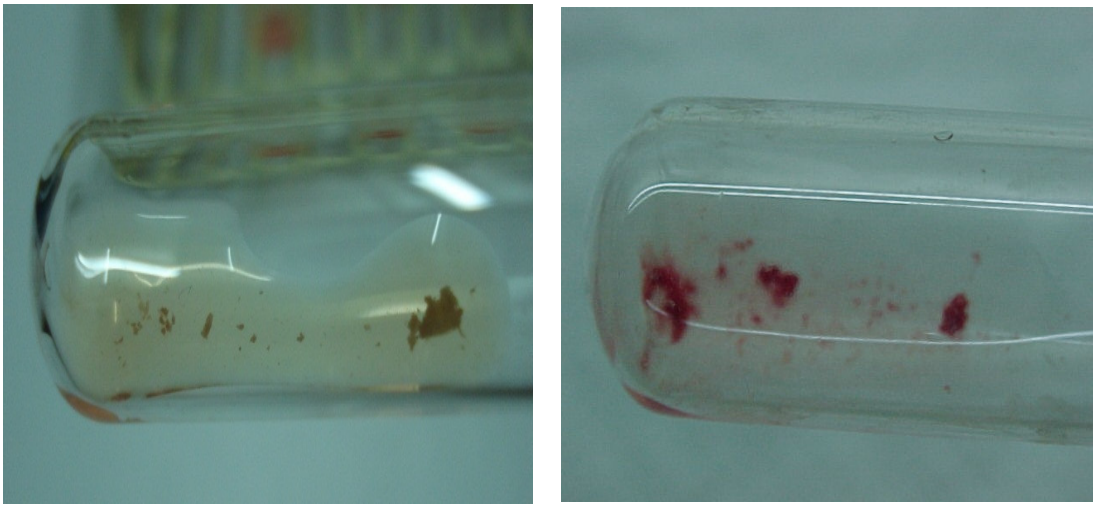
The processing consisted of applying the CD44 dosage technique by measuring the inhibition of the adhesion or agglutination. In the agglutination inhibition reaction, the specificity and antibody titre estimation was performed based on competition between soluble and particulate antigens for antibody combining sites. This might be equiparated to the Hyalunorate Receiver reaction (CD44-Hyaluronate). In these reactions, first the ligand is combined with the soluble receptor, inhibiting then the indirect adhesion (agglutination) of cell indicator. It was interpreted that in the presence of CD44, hyaluronic acid was consumed, leading to lower erythrocytes aggregation. *(D'Arrigo et al, 2009)*

The technique consists in initially added one and a half volume of bromelain at a 5mg/ml concentration, at "Coulot" volume of red blood cells and then incubated for 15 min at 37 ° C. Then the RBCs were washed and centrifuged for 3 minutes at 3000 RPM. After this process, the supernatant was

removed with an aspiration pipette and it was suspended in phosphate buffered saline (PBS) at 2% *(Figure 4)*. Meanwhile 11 clean tubes were labelled as "pure", "1/2", "1/4", "1/8", "1/16", "1/32", "1/64", "1 / 128 "," 1/256 ", 1/512" and "1/1024", respectively.

The pulp sample to be study was taken the most ground possible. This pulp was added to the first tube labelled ("pure") and to the second ("1/2"). Then the geometric dilution was performed: the entire volume of the 2nd tube ("1/2") was taken and a drop was added to the 3 ("1/4"), returning to the original tube the leftovers, and so on until the last tube, completing the whole series and having the same volume of liquid in all tubes. From 2 ° to 11 ° tube a drop of BPS was added (pipette deposit in the bottom of the tube). In each of the 11 labelled tubes a drop of hyaluronate was poured and they were taken to incubate in a refrigerator for 15 minutes at 4 ° C. A dilution of the serum of the patient of the sample same group was done to be used as control.

In a separate tube 6 drops of washed red cells and 9 drops of bromelain were mixed, placing them in a clean tube. This tube was incubated for 15 min at 37 ° C. A drop of bromeliaceous red cell was poured in each of the labelled tubes in which the geometric dilution was done, even in the 1st tube ("pure") and they were incubated 24 hours in the refrigerator; after this time the reading was made observing agglutinated titration. *(Figure 5)*



Reaction (-) Reaction (+++)

Figure 5. Agglutination after 24 hours of incubation

The titrations results were expressed in scores. *(Table 1)*

Score 10:	+ + + (> 10 large agglutinated)
Score 8:	+ + (3 to10 agglutinated)
Score 5:	+ (1 to 3 agglutinated)
Score 2:	± (trace)
Score 0:	- (without agglutinated)

Table 1. Scores assigned according to the number of agglutinated

Source: Rasia Valverde JR, Rasia RJ. Low ionic strength solutions in the Coombs test to detectanti-Rh antibodies.
Latinoamerican Clinical Biochemistry act 1982, 16 (2): 297-305.

Statistical analysis.

The results of the titrations expressed by a score were applied in a mathematical expression called "sensitivity parameter" *(Valverde and Rasia, 1982)* that combines tittre and degree of dilution, symbolized by *α* and estimated as:

$$\alpha = \sum_{i=1}^{n} (E_i / D_i) 10^{-3}$$

Where:

E i = Score of erythrocyte adhesion degree
D i = serum dilution.

The estimation of the α value was calculated for each sample of pulp tissue, varying the titre (represented by score) and the respective level of dilution; this parameter allowed to quantify, more accurately, the increased sensitivity of a specific technique. The relationship between the patient age with the appropriate sensitivity parameter was studied using the Spearman's Rank Correlation Coefficient; its statistical significance was measured in terms of whether or not the value 0 (zero correlation) was included in the respective 95% confidence interval. To evaluate the existence of differences between the medians of the parameter sensitivity of the considered subgroups it was applied the Kruskall-Wallis; to identify which subgroups differed among themselves the Mann-Whitney test was applied. In all statistical tests, a $P<0.05$ value was considered statistically significant.

RESULTS

28 samples of dental pulp from 25 patients (12 males and 13 females) between 2 and 13 years old were evaluated.

Patients s group and blood factor were evaluated, resulting that more than half had the group A + (16 patients, 64%), followed by 0 + group (7 patients, 32%); only 1 patient had group B + (4%).

Considering the origin of the pulp, 22 samples were from decidious teeth (10 with healthy pulp, 12 with pulp with caries) and 6 came from supernumerary teeth. The pulp obtained in each of the samples of the different groups was subjected to a biochemical technique for detecting the presence of CD44 with the technique described before.

Table 2 shows an example of how the data were originally collected: teeth group owners, age, sex, blood group of material donor, and then the erythrocyte adhesion degree observed for each level of dilution.

GROUP	TOOTH	AGE	SEX	BLOOD GROUP	Dilution level (D)										
					PURE	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
healthy pulp	84	10	M	A +	-	-	+	+	+	+	+	±	+	-	-
healthy pulp	63	12	M	A +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +
c pulp / tooth	53	9	F	A +	-	±	±	±	±	-	-	-	+	±	
healthy pulp	53	11	F	0 +	+	±	+ + +	+ + +	+ +	+ +	+	+ +	+ +	+ +	
supernumer	52	10	F	0 +	+ + +	+	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	
c pulp / tooth	65	12	F	0 +	+	+ +	+	+	+ +	+	-	+	-	-	+
healthy pulp	85	11	F	A +	+ +	+	+ + +	+ +	+ +	+	+ +	+ +	+	+	+
supernumer	53	8	F	A +	±	±	±	-	+	+ + +	+ +	+	±	+ +	+ +
healthy pulp	75	10	M	0 +	+	+	+ +	+ +	+ +	+ + +	+ +	+ + +	+ +	+ +	+
healthy pulp	64	11	M	A +		±	±	+	±	±	+	±	+	+ +	+

Table 2. Identification of the sample and erythrocyte adhesion degree to each level of dilution of pulp types tested. Tabulating model of the data under study.

Table 3 shows the above same table, but replacing the degree of agglutination obtained in each sample by the respective scores mentioned before. On the right, the sensitivity parameter calculated for each sample.

GROUP	TOOTH	AGE	SEX	BLOOD GROUP	Dilution level (D)											α
					PURE	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	
healthy pulp	84	10	M	A +	0	0	5	5	5	5	5	2	5	0	0	0.00246
healthy pulp	63	12	M	A +	8	8	8	8	10	10	8	10	10	10	10	0.00821
c pulp / tooth	53	9	F	A +	0	2	2	2	2	0	0	0	5	2	0	0.00190
healthy pulp	53	11	F	0 +	5	2	10	10	8	8	5	8	8	8	0	0.00569
supernumer	52	10	F	0 +	10	5	5	10	10	10	10	10	10	10	0	0.00623
c pulp / tooth	65	12	F	0 +	5	8	5	5	8	5	0	5	0	0	5	0.00658
healthy pulp	85	11	F	A +	8	5	10	8	8	5	8	8	5	5	5	0.00688
supernumer	53	8	F	A +	2	2	2	0	5	10	8	5	2	8	8	0.00232
healthy pulp	75	10	M	0 +	5	5	8	8	8	10	8	10	8	8	5	0.00657
healthy pulp	64	11	M	A +	0	2	2	5	2	2	5	2	5	8	5	0.00245

Table 3. - Identification, erythrocyte adhesion score for each level of dilution and parameter sensitivity in the different types of evaluated dental pulp

Age relationship at tooth involutive different stages

Figure 6 shows the influence of age in tooth involutive stages. Age showed a statistically significant relationship with CD44 concentration (Spearman coefficient: 0.4344, CI95%: 0.0615 to 0.7008).

The age influence was more evident in the agglutination titres in the supernumerary teeth pulp. Indeed, erythrocyte agglutination, indicative of CD44 presence, was markedly higher in these pulp parts (yellow rhombus), which remained sensitivity values between 0.008 and 0.01, while in the remaining patients mostly with deciduous teeth showed lower values in agglutination titres.

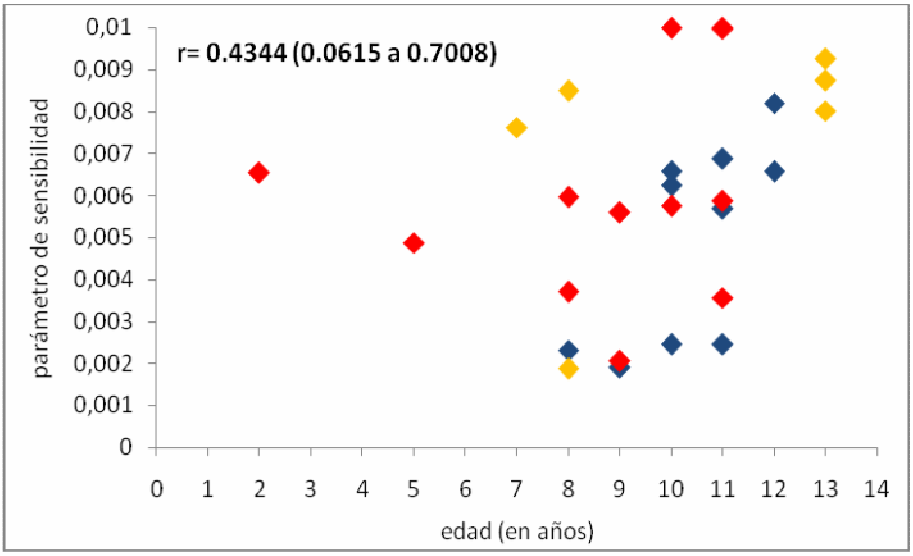


Figure 6.
Erythrocyte adhesion parameter sensitivity in relation to the patient's age

In blue n = 10 samples of healthy deciduous pulp.
In red, n = 12 samples of deciduous pulp with caries.
Yellow n = 6 supernumerary pulp samples

Sex relationship at tooth involutive different stages

Evidence suggested that sex had no influence on the involutional stages of the tooth. *Figure 7* shows that sex was not related to CD44 levels detected in the patient's teeth pulp (Spearman coefficient: 0.3582, CI 95% -0.02888 to 0.6518).

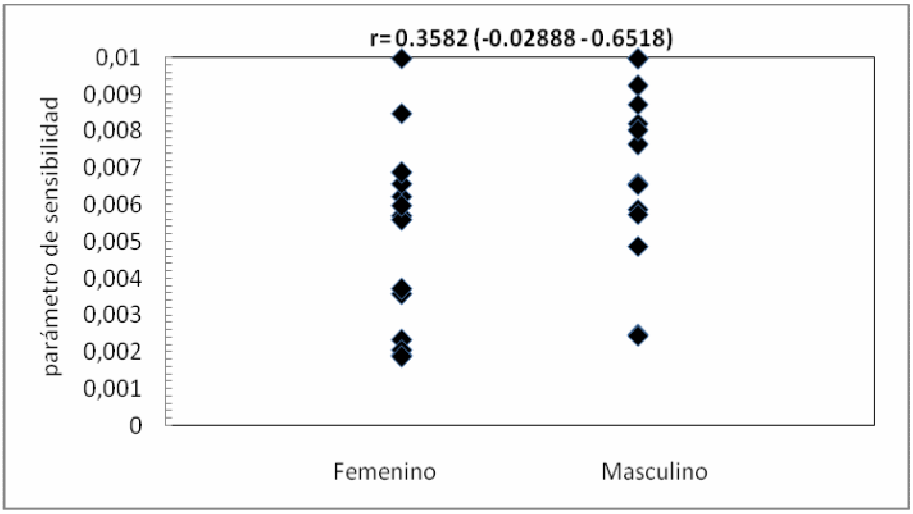


Figure 7. Parameter sensitivity of erythrocyte adhesion in relation to patient sex

Relationship of blood group in involutive different tooth stages

Figure 8 shows that the patient blood type (group and factor) had no influence on the tooth involution. The evidence showed that the blood group and factor did not correlate with CD44 levels detected in the patient's dental pulp (Spearman coefficient: -0.1238; CI 95% : -0.4840 to 0.2722).

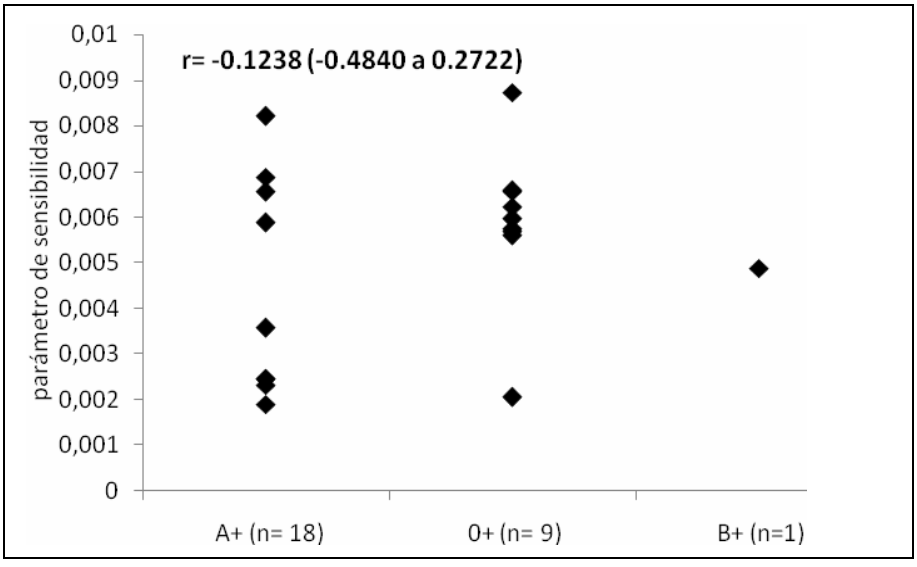


Figure 8.
Sensitivity parameter of erythrocyte adhesion in relation to the patient's blood group and factor

Comparative analysis of the groups

In the healthy pulp group (n = 10), the α sensitivity parameter average was 0.00493, with a range that varied between 0.00190 and 0.00821. The median value was 0.00596.

In the pulp with caries (n = 12) group, the α sensitivity parameter average was 0.00606, with a range that varied between 0.00206 and 0.00999. The median value was 0.00575.

In the group of supernumerary teeth (n = 6), the α sensitivity parameter average was 0.00742, with a range that varied between 0.00188 and 0.00938. The median value was 0.00819.

The degree of CD44 concentration shows differences among the analysed groups but with a *borderline* statistical significance (P=0.0583 of Kruskal-Wallis test) *(Figure 9)*. The intense concentration variability within and between the evaluated groups demanded a specific analysis between them.

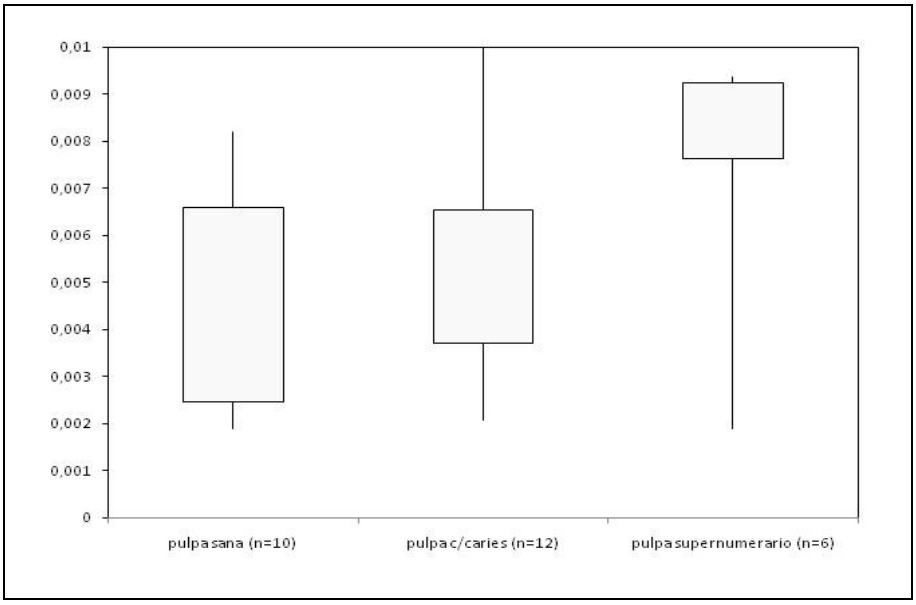


Figure 9. Erythrocyte adhesion sensitivity parameter in the analysed groups

From the first two boxplot, it was found that the pulp of healthy deciduous teeth showed similar concentration of CD44 that samples from deciduous teeth with caries (P = 0.6277 of Mann-Whitney test).

When compared the CD44 concentration in healthy deciduous pulp and in supernumerary pulp, statistical evaluation suggested that the degree of agglutination in the pulp of supernumerary teeth was significantly higher than the reached in the group with healthy pulp (P = 0.0559 of Mann-Whitney test).

Moreover, the sensitivity parameter did not reach statistically significant differences in the group with pulp with caries, both in the temporary group and in the healthy pulp group (P = 0.4371 of Mann-Whitney test).

DISCUSSION

This thesis was done for studying the distribution of CD44 adhesion molecule in dental pulp tissue to establish the expression pattern under *in vitro* normal conditions compared with pathological (decay processes) and physiological (supernumerary teeth) conditions. The technique used in this work, of qualitative measurement, can perfectly be replicated with quantitative measurement techniques (eg ELISA) to determine CD44 concentration.

It was noted that the α parameter as an indicator of the CD44 concentration in dental pulp tissue, provided an excellent system to study the histochemical changes in the physiological resorption process of deciduous teeth.

Osteoclastogenesis is an inflammatory process effect established on a mineralized tissue. This has been suggested by observing, among other things, that the pro-inflammatory cytokines are essentially the same than the pro-resorptive cytokines and that the only thing that varies is the type of tissue where inflammation settles. Inflammatory processes, as well as other biological functions, require the interaction of adhesion molecules in events signalling through the cell membrane (from outside to inside and vice versa); they are governed under different time rhythms, which goes from seconds to days. The knowledge of the regulation of these molecules expression, their activation state on the tooth surface, the cell and tissue distribution and their possible interactions, are of crucial importance to understand the mechanisms of action involved in the root resorption process.

Sex and blood type and factor of each patient do not showed statistically significant evidence of correlation with CD44 concentration in the dental pulp of the various groups evaluated.

It was observed that the CD44 concentration increased with older ages, consistent with the increased of resorptive activity.

A first analysis showed that the CD44 concentration level was statistically similar in healthy and decayed pulp samples. It is assumed that this similarity was because both groups patients were in the physiological resorption process; the average age in the group with deciduous teeth healthy pulp (10.4 years, 8-12 years old range) coincides with the eruption process of the canine and first permanent mandibular and maxillary premolar; the deciduous teeth roots showed 2/3 reabsorbed. The average age of the group with deciduous teeth pulp with caries (8.8 years old, range: 5-11 years) represents the final stage of the first period of replacement of primary for permanent dental formula; the deciduous teeth roots have been completely reabsorbed.

The CD44 concentration was significantly higher in supernumerary teeth. The evidence showed that the dental pulp of permanent supernumerary had an increase of over 50% in the average of CD44 concentration in comparison with the registered on healthy teeth pulp, and an increase of over 22% from what was

measured in the pulp of tooth with caries. In molecular terms, these findings suggest that the hyaluronate receptor molecule is present in a more considerable manner in dental pulps without resorption processes.

Historically the supernumerary dentition phenomenon was reserved mainly for males, but prevalence studies, which included a review of more than 8000 X-rays, noted that it is more common in females. *(Kaya et al, 2011)* Other authors suggested that the location pattern of these teeth depends on gender; pieces in the midline region occur more frequently in men, while in the incisal region are more common in women. *(Küchler et al, 2011)* Although supernumerary teeth epidemiology has been well studied, the exact genetic etiology and molecular mechanisms that promote this kind of teeth has not been completely clarified yet. Their different morphologies, the affected regions and the development process of these teeth become the support of several theories about this condition. One theory suggests that the temporary is created as a result of a dichotomy of the tooth bud. Another theory, well supported in literature, is the hyperactivity theory, which suggests that this tooth is made up of the dental lamina local hyperactivity. It has also been suggested that they are a result of atavism, meaning a reversion to a more primitive type of dentition. However atavism cannot explain the presence of supernumerary in the canine and midline regions or tuberculate forms in any region. Heredity may also play a role in the occurrence of this anomaly, and supernumerary are more common in the relatives of affected children than in the general population. Mutant genes could also account for this anomaly. *(Liu, 1995; Garvey, Barry and Blake, 1999; Küchler et al, 2011)*

Currently, a number of models with rats allow the investigation of the complex genetics of supernumerary teeth. Many of the known sequences of molecular signalling involved in the normal development of the tooth germ can generate an extra teeth if they are improperly regulated; this includes fibroblast growth factor, tumour necrosis factor, WNT and osteogenic proteins, which provide candidate genes that can potentially play a role in the formation of human supernumerary teeth. *(Fleming et al, 2010)*

The coordinated regulation of the molecular signalling activity is particularly important to ensure proper dental number and there are different requirements for this suppression in epithelial and mesenchymal tissues, both along the axes of the jaws and between the jaws themselves. In mouse models a number of fundamental mechanisms which facilitate the formation of supernumerary teeth have been shown. A key process appears to be the early death of the vestigial tooth primordia present in the embryo, achieved through the suppression of the signalling pathway of development (also called SHH) within these early teeth. However, restriction of WNT signalling is also important in controlling tooth number, with increased transduction being capable of generating multiple tooth buds from the oral epithelium or existing teeth themselves, in both embryonic and adult tissues. Indeed, the uncontrolled activity of this pathway can lead to the formation of odontogenic tumours containing multiple odontogenic tissues and poorly formed teeth. Disrupted patterning along the buccal-lingual aspect of the jaw can produce extra teeth directly from the oral epithelium in a duplicate row. Together, all these

findings have relevance for human populations, where supernumerary teeth are seen in association with both primary and permanent dentition. *(Cobourne and Sharpe, 2010)*

Recent molecular studies with these teeth revealed that interleukin-11 signalling is essential for normal development of craniofacial bones and teeth, and that its function is to control their numbers, these results open the possibility of regulating the signaling of this protein. *(Nieminen et al, 2011)*

There were found no published studies that measure the activity of the CD44 molecule in supernumerary teeth in order to compare experiences, but the evidence found in this thesis confirms what was published, that the CD44 molecule plays a role in tissue regeneration. *(Laino and et al, 2006; Andreasen and Lovschall, 2010; Hirata et al, 2010)*

Tissue engineering is the science of design and manufacture of new tissues. Deciduous and supernumerary teeth may be a significant source of stem cells, which with the involvement of tissue engineering, help to repair damaged tooth structures, to induce bone regeneration and tissue reconstructions treatment, sometimes even of large dimensions such as that of mandibular body and *condyle.* Advances in stem / progenitor cells of dental pulp could give an impulse to regenerate dentin tissue without removing all the pulp.

A recently published study that reports the isolation of stem cells of deciduous teeth pulp related cell proliferation to the physiological process of resorption of the tooth from where the pulp was extracted. Different markers, examined using flow cytometry, including CD44 were used. Although the study focused on the kinetics analysis of cell production *in* vitro, it was found that the CD44 concentration remains highly positive in cytometric cell cultures. *(Bernardi et al, 2011)*

In general, human tissues have a very limited regeneration potential. However, recent advances in stem cell research and tissue engineering promise new prospects for tissues regeneration in dental practice in the future. Stem cells have been discovered in many adult tissues, including teeth, and embryonic stem cells have the potential to form all adult tissues. With the rapid advances in molecular biology, it is almost time for developing enamel, dentin, periodontal ligament, bone or teeth, completely new for patients in the future. *(Thesleff and Tummers, 2003)*

Due with studies of the past 20 years tooth development is now beginning to be understood at the gene level. There were genes identified with key roles in mediating cell communication, considered the most important mechanism of embryonic development; this communication is mediated by a large number of different signaling molecules, which are sent to the surroundings of the surface of the cell to the contact with their respective receivers forming a complex signaling network. Combining the knowledge of the molecular regulation of tooth development with advances in stem cell research, the focus is on the potential applications of tissue regeneration in dental practice. *(Thesleff, 2003)*

Scientific advances in the development of restorative biomaterials, the technology of in vitro cell culture, tissue grafting, tissue engineering, molecular biology and the human genome project are the basis for the introduction of new technologies in dentistry. It has already been shown to mineralize teeth in response to injury, but only in recent years it has been localized the stem cells position round blood vessels. The cells have been identified as pericytes miofibroblastoides.

The ability to control the differentiation and proliferation of these cells is being examined to create cell therapies that can solve dental problems more effecienty than current treatment regimens. (*Murray and Garcia Godoy, 2004)*

Research by *Miura et al* *(2003, 2006)* gave evidence that stem cells from deciduous teeth represent a population of postnatal stem cells able of reproduce and multipotent differentiate themselves. Deciduous teeth can be a significant source of these cells, which with tissue engineering intervention, allow to repair damaged tooth structures, to induce bone regeneration and tissue reconstructions to be treated, sometimes even of large parts such as the mandibular and condyle

It have also been isolated and characterized stem cells from a supernumerary tooth, rescuing the possibility of obtaining these cells in pulps of teeth which are usually discarded. *(Huang, Chen et al, 2008*)

Departing from a new paradigm in the research, *Huang, Snyder et al* *(2008)* grafted dental stem cells without differentiating of rhesus monkeys in the mice inmunodeficient hippocampus. These authors reported that the implant stimulated endogenous neuronal cells proliferation and pre-existing neural stem cells were detected and also mature neurons in the graft site; they also referred that the implant promoted the growth signaling factor to one month after the implantation. They confirmed that the stem cells of the dental pulp have a valuable and unique therapeutic potential, and in the case they described a potential stimulator and modulator of the local repair in the central nervous system response; they concluded their investigation stating that dental stem cells would be a source "preferable" to others, avoiding the problems associated with graft rejection by immunological causes.

In the last decade, researches on possible applications of stem cells in dentistry have made great advances. There are at least five different types of multipotent mesenchymal stromal cells (MSCs). It has been reported that the MSCs derived from dental tissue are capable of generating complex as the pulp-dentine as well as differentiate into periodontal and craniofacial progenitor cells. Moreover, from the adult stem cells, the embryonic stem cells are an alternative source of cells for dental tissue regeneration. Besides these commonly reported stem cells, other progenitor cells with MSC properties are also found in salivary glands, in the muscle of the tongue, palate and buccal mucosa, and these may play a role in the recovery of tissue function which reside. (*Liu and Cao, 2010*)

CONCLUSION

CD44 is a transmembrane glycoprotein involved in adhesion between cells and various extracellular matrix components. Its principal ligand is the hyaluronate, although others ligands such as laminin, fibronectin and collagen type I and IV may also be involved.

CD44 is involved in cell motility and migration by adhering to extracellular matrix molecules. It has a broad tissue distribution, especially in leukocytes and erythrocytes. Recent studies in both experimental animals and in human tumours have involved CD44 expression in different tumour biology.

It was found in this study that the concentration of CD44 in primary teeth increase according to the children age, consistent with resorptive activity increased in rhizolysis processes on teeth of primary dentition.

It was demonstrated that the level of CD44 concentration was statistically similar in the healthy and decayed deciduous pulp samples, due to the fact that the teeth of both groups were in the physiological resorption process (average age: 10.4 and 8.8 years old, respectively). The concentration of CD44 in supernumerary permanent teeth pulp samples was significantly higher than that the registered in deciduous pulp with or without cavities. In molecular terms, these findings suggest that the hyaluronate receptor molecule is more substantially present in dental pulps without resorption processes. This evidence confirms all that was published, that the CD44 molecule plays a role in tissue regeneration.