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Effects of hydroxyapatite associated to collagen type I graft in bone repair of critical defects in rabbits

Efeitos da hidroxiapatita associada ao enxerto de colágeno tipo I no reparo ósseo de defeitos críticos em coelhos

Efectos de la hidroxiapatita asociada al injerto de colágeno tipo I en la reparación ósea de defectos críticos en conejos

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Abstract
The aim of this investigation was to evaluate the influences of a malleable bone graft composed by hydroxyapatite and collagen type I in bone formation. Three surgical sites of critical size were prepared in twelve rabbits' calvarias. The control defect was fulfilled with blood clot and the experimental defects were fulfilled with the graft. The euthanasia occurred in three times: 4, 8 and 12 weeks after the surgical procedures. The specimens were analyzed in light, fluorescence and scanning electronic microscopes. The quantitative evaluation was made by histomorphometric analysis, in order to calculate the percentage of new bone formed and the area of the hydroxyapatite particles. The results were submitted to two way ANOVA test and Tukey`s post hoc. New bone formation was accelerated in early stages of the healing process. It was seen more intensive bone formation in the periosteum side than in the dura mater membrane side in all groups. Besides that, in experimental sides the new bone was surrounding the hydroxyapatite particles with minimal amount of inflammatory cells, which confirmed the biocompatibility and osteoconduction properties of this material. Besides that, the hydroxyapatite particles showed gradual and progressive absorption at the same time that the bone was being formed.
Keywords: Hydroxyapatite; Collagen; Boner.

Resumo
O objetivo desta investigação foi avaliar as influências de um enxerto ósseo maleável composto por hidroxiapatita e colágeno tipo I na formação óssea. Três sítios cirúrgicos de tamanho crítico foram preparados em doze calvárias de coelhos. O defeito controle foi preenchido com coágulo sanguíneo e os defeitos experimentais foram preenchidos com o enxerto. A eutanásia ocorreu em três momentos: 4, 8 e 12 semanas após os procedimentos cirúrgicos. Os espécimes foram analisados em microscópios eletrônicos de luz, fluorescência e varredura. A avaliação quantitativa foi feita por análise histomorfométrica, a fim de calcular a porcentagem de osso novo formado e a área das partículas de

hidroxiapatita. Os resultados foram submetidos ao teste ANOVA de duas vias e post hoc de Tukey. A nova formação óssea foi acelerada nos estágios iniciais do processo de cicatrização. Foi observada formação óssea mais intensa no lado do periósteo do que no lado da membrana da dura-máter em todos os grupos. Além disso, nas vertentes experimentais o novo osso circundava as partículas de hidroxiapatita com quantidade mínima de células inflamatórias, o que confirmava a biocompatibilidade e as propriedades de osteocondução deste material. Além disso, as partículas de hidroxiapatita apresentaram absorção gradual e progressiva ao mesmo tempo em que o osso estava sendo formado.
Palavras-chave: Hidroxiapatita; Colágeno; Osso.

Resumen
El objetivo de esta investigación fue evaluar las influencias de un injerto óseo maleable compuesto por hidroxiapatita y colágeno tipo I en la formación ósea. Se prepararon tres sitios quirúrgicos de tamaño crítico en doce calvarias de conejos. El defecto de control se cumplió con el coágulo de sangre y los defectos experimentales se cumplieron con el injerto. La eutanasia ocurrió en tres tiempos: 4, 8 y 12 semanas después de los procedimientos quirúrgicos. Los especímenes fueron analizados en microscopios electrónicos de luz, fluorescencia y barrido. La evaluación cuantitativa se hizo por análisis histomorfométrico, para calcular el porcentaje de hueso nuevo formado y el área de las partículas de hidroxiapatita. Los resultados fueron sometidos a la prueba ANOVA de dos vías y post hoc de Tukey. La formación de hueso nuevo se aceleró en las primeras etapas del proceso de curación. Se observó una formación ósea más intensa en el lado del periostio que en el lado de la membrana de la duramadre en todos los grupos. Además, en los lados experimentales, el hueso nuevo rodeaba las partículas de hidroxiapatita con una cantidad mínima de células inflamatorias, lo que confirmó las propiedades de biocompatibilidad y osteoconducción de este material. Además de eso, las partículas de hidroxiapatita mostraron una absorción gradual y progresiva al mismo tiempo que se iba formando el hueso.
Palabras clave: Hidroxiapatita; Colágeno; Hueso.

1. Introduction

Reconstruction of bone defects caused by tumor, periodontal disease, dental extraction, trauma, cleft palate and others is a major issue in maxillofacial surgery. Several graft materials have been used to improve bone topography. It can be autografts, allografts, xenografts and synthetic composites (Benic, et al. 2022, Janjua, et al. 2022, Lu, et al. 2022).

Effectiveness of a bone graft can be described by three biological properties: osteoinduction, osteoconduction and osteogenesis (Bauer & Smith, 2002, Chalmers et al. 1975, Faundez, et al. 2006, Urist, 1965). The only bone graft that presents these characteristics simultaneously is autogenous bone, being considered as the gold standard once it contains live cells and growth factors (Chase & Herndon, 1955, Faundez, et al. 2006, Giannoudis, et al. 2005, Goldberg & Stevenson, 1987, Parrish 1966, Wong, et al. 2002). However, the long-term morbidity due to autogenous bone harvesting can be as high as 30% (Faundez et al. 2006). Beyond the donor site morbidity, autogenous bone graft has problems as variable resorption leading to loss of bony edge overtime and difficulty to be adjusted to the desired shape into the receptor site (Hsu, et al. 2005).

Ceramics calcium-based materials such as tricalcium phosphate, hydroxyapatite (HA), and biphasic mixtures of both components are considered too much promising substances (Arts, J.J., Gardeniers, J.W., et al. 2005) and have been studied since 1970 (Gosainet al. 2002). These materials are advantageous because of their unlimited availability, as well as granule production of every size and porosity (Arts, et al. 2005). Ceramics calcium-based materials have also biocompatibility without eliciting an inflammatory or foreign body response, as well as osteoconduction properties (Fujishiro, et al. 2005, Jarcho, M. 1992). Experimental studies revealed HA osteoinduction potential after subcutaneous implantation of HA-derived biomaterials in different animal models (Gosain, et al. 2005, Gosain, et al. 2002, Ripamonti, U. 1996).

Mineral bone produced from bovines is a natural microporous HA (Oliveira, et al. 2003) and is frequently used as graft material because of its specific surface that resembles cancellous bone, in addition to the complete deproteinization of inorganic component and thus absence of antigenicity (Haas, et al. 1998).

Problems associated to HA use include containment of HA particles during oral surgery and maintenance of the material on the ridge in the postoperative period (Mehlisch, D.R. 1989, Taylor & Helfrick, 1989). In order to eliminate these mobility, HA is often mixed with collagen, gelatin and fibrin glue (Hsu, F.Y., Tsai, S.W., et al. 2005) or autogenous graft

(Mehlisch, 1989). The HA can be associated to collagen type I, which has properties such as support the orientation of blood vessels and creation of new Haversian systems into the bone scaffold (Giannoudis, et al. 2005). This graft material may act as a valid bone substitute under favorable conditions, in the presence of bone marrow cells, healthy bleeding bone and mechanical stability (Faundez et al. 2006).

Collagen contributes to mineral deposition, vascular ingrowth, and growth factor binding, providing a suitable environment to bone regeneration. It is not advantageous material as bone graft by itself, but once coupled with another material as HA, collagen enhances significantly graft incorporation (Giannoudis, et al. 2005). Collagen is a component of the extracellular matrix functioning as cell adhesive and inducing osteoblasts proliferation, which should accelerate bone regeneration (Hsu et al. 2005, Rodrigues et al. 2003). It might be expected that additional collagen promotes aggregation, adhesion and proliferation of cells as well as regulates orientation and proliferation of fibroblasts, making a collagenous fiber matrix (Ishikawa et al. 2001, Minabe et al. 1988, Wiedmann-Al-Ahmad et al. 2005).

In accordance to the literature concerning the ceramics calcium-based materials used as bone grafts, the aim of this study was to evaluate the bone repair of critical defects with a composite of hydroxyapatite/collagen type I graft.

2. Methodology

This research was performed with 12 adult male New Zealand White Rabbits weighing 2.700 to 3.000 g. The experimental protocol was approved by the Ethical Committee for Animal Research (CAUAP), at Federal University of Rio de Janeiro and was performed according to the standards of the Brazilian College of Animal Experimentation (COBEA). The animals were kept in individual cages with pellet food and water ad libitum.

After 30 days of acclimatization, all the animals were submitted to an even surgical protocol to create bone defects in the calvaria, being grouped according to the euthanasia time after surgery, as it follows 4 weeks (Group 1), 8 weeks (Group 2) and 12 weeks (Group 3), each one with 4 rabbits.

Prior to surgery, the animals were anesthetized by an intramuscular injection of ketamin (Ketalar® - Pfizer) 50 mg/kg of body weight associated to xylazine (Rompum® - Bayer) 5 mg/kg of body weight.

The calvaria was shaved and disinfected prior to incision. Infiltration of the surgical site was performed using 0,5 ml of mepivacaine 2% with epinephrine 1:100.000 to reduce bleeding.

2.1 Surgery

A midline incision with 6 cm of extent was made through skin and periosteum in order to expose calvaria`s bone, using as anterior reference the average distance between the orbits and the ear's insertion as posterior reference. Three cranial orifices with critical size (Kramer, I.R.H., Killey, H.C., et al. 1968), that is, defects with dimension of difficult healing, were created using a trephine drill (diameter of 8 mm) under low rotation and saline irrigation to avoid thermal damage. One defect was located along the midline (Control Defect) and the two others adjacent to the left and right sides of it (Experimental Defects), in posterior region to the control defect. The underlying dura mater membrane was carefully preserved. The graft of hydroxyapatite and collagen type I then placed into the two experimental defects. The midline defect was fulfilled with blood prior to suture.

The graft used in this experiment was composed of bovine hydroxyapatite (75%), that is a bovine deproteinized cancellous bone with a structure similar to human bone, and bovine collagen type I (25%), presenting the same physical and chemical characteristics as seen in human collagen fibers. Both substances were processed and sterilized by gama radiation. This material was available in a lightly malleable cylindrical form with 1 g, 2 g, 6 g, 10 g, 15 g and 20 g.

The soft tissue was closed in layers in order to promote additional protection to the calvarium defects. For this, it was used reabsorbed sutures in periosteum and muscles as well as silk thread suture in skin.

2.2 Postoperative Period

It was administered a single intramuscular injection of benzil penicillin benzatine, 40.000 IU/kg of body weight (Benzetacil®) just after the surgery and an anti-inflammatory (Banamine® – Shering-Plough) by a subcutaneous injection of 1,1 mg/kg of body weight for 3 days, to avoid postoperative infection and inflammation.

The polyfluorochrome sequential labelling was carried out to assess the new bone formation and remodeling pattern in the experimental and control defects. The animals received intramuscular administration of tetracycline (Ourotetra L.A.), alizarin (Sigma-Alderich) and xylenol orange (Sigma-Alderich) on the fifth day after the surgical procedures and at each 10 days until the euthanasia (Table 1). The alizarin and xilenol orange were associated to a sodium phosphate dibasic solution (Na2HPO4).

Table 1. Doses of the bony fluorescent markers used in the experiment.

BONY MARKERS	DOSES	INJECTION
OXITETRACYCLINE	10mg/kg	20mg/50ml
XYLENOL ORANGE	90mg/kg	6g/100ml + 2g Na2HPO4
ALIZARIN	30mg/kg	3g/100ml + 2g Na2HPO4

Source: Authors.

The animals were anesthetized and the euthanasia was performed by intracardiac injection of potassium chloride solution, 4, 8 and 12 weeks after surgical procedures according to the experimental protocol. The anatomical specimens of interest were dissected.

2.3 Descriptive Analysis

For histological observation in light microscopy, the dissected tissue was fixed in Bouin solution during 24 hours. The specimens were decalcified by 5% trichloroacetic acid. After that, they were dehydrated in graded series of alcohol concentration from 50-100%, embedded in paraffin and sectioned in transversal direction to the bony defect. Sections of 6 μm thickness were stained with hematoxylin and eosin for descriptive analysis of the defect areas and its adjacent tissues under light microscope (HM-LUX E600 - Nikon).

The specimens derived from animals whose received oxitetracycline, alizarin and xylenol orange administration were fixed in paraformoldeyde during 24 hours. Then, they were dehydrated, included in Epoxy (Spurr's médium) and polymerized for 24 hours, sectioned with 70 μm thickness in the same direction as the decalcified ones. The ground sections were polished and analyzed at Axioskop2 Plus microscope with Fluorescein Fs 09 filter.

The anatomical specimens analyzed under scanning electronic microscopy (SEM), were fixed in formalin, dehydrated, dried in a Baltec CPD-030 Critical Point Dryer and coated with gold. The analysis was performed in a JSM-6360LV Scanning Electronic Microscope.

2.4 Histomorphometric Analysis

Histomorphometric evaluation was performed in decalcified histological sections of the control and experimental bony defects. Four digital photographs of five sections, of each control and experimental surgical sites, were made at

magnification of 10x, in order to obtain its total extension. The sections were then assessed in the Image-pro Plus 4.5 program with a grid mask in order to count the percentage of bone, connective tissue, hydroxyapatite, blood vessels and medullar spaces in this respective area (Jensen, S.S., Broggine, N., et al. 2006). In the central region of each site, it was also measured the area occupied by the hydroxyapatite particles of the graft material.

2.5 Data Analysis

Statistical analysis of the histomorphometric data was performed to compare the Groups of 4, 8, 12 weeks, using analysis of variance (two way ANOVA) and post-hoc Tukey test, using SPSS software (version 13.0). The significance level for all tests was ≤ 0.05.

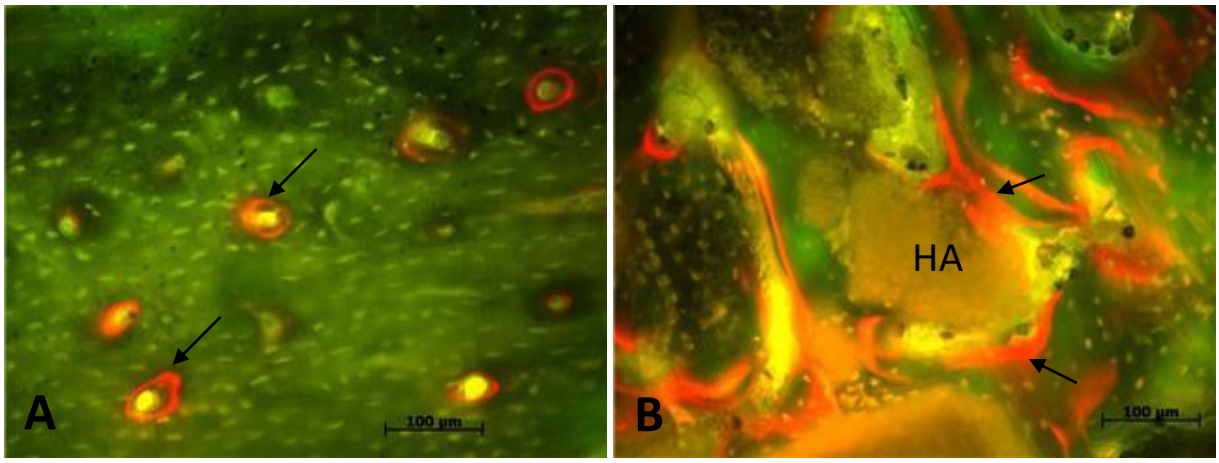
3. Results

All the animals of Groups 1, 2 and 3 survived their respective experimental period of 4, 8 and 12 weeks. None of them had postoperative complications or infections. It was observed satisfactory clinical evolution in the control and experimental calvarias surgical defects. A progressive increase of new bone formation occurred in agreement with time in control defects as well as in the ones that received the graft material. In both of them, the beginning of the new bone formation was more intensive in the periosteum side than in the dura mater membrane side of the defect. It was seen a tenuous amount of inflammatory cells at 4, 8 and 12 weeks of the experiment. The thickness in the experimental defects was kept in all groups.

3.1 Group 1 – 4 weeks

The new bone formation could not be detected at this early stage of repair in the control defects unless by few and small islands of bone in an extensive amount of connective tissue (Figure 2A). It was seen the line of osteoblasts along the interface of the defect adjacent to the host bone. In the experimental defects, most of them was filled with hydroxyapatite particles encased in a fibrous connective tissue network (Figure 2B). Some particles were surrounded by new bone and there was great amount of blood vessels. This finding was also evident in the fluorescence microscopy analysis at 4 weeks, by the signal of bone formation around of the HA particles, as well as seen in the other experimental periods (Figure 3). In the SEM, it was observed collagen fibers in both defects and some osteoblasts fixed in hydroxyapatite particles of the experimental defects like in Groups 2 and 3 too (Figure 2).

Figure 2 - Photomicrographs of the ground sections relative to specimens with 8 weeks. A Control site, illustrating isolated areas of bone formation in concentric lamellas (arrows). B Experimental site, exhibiting bone formation (arrows) around of the hydroxyapatite particle (HA). Fluorescence. Scale bar = 100 µm.

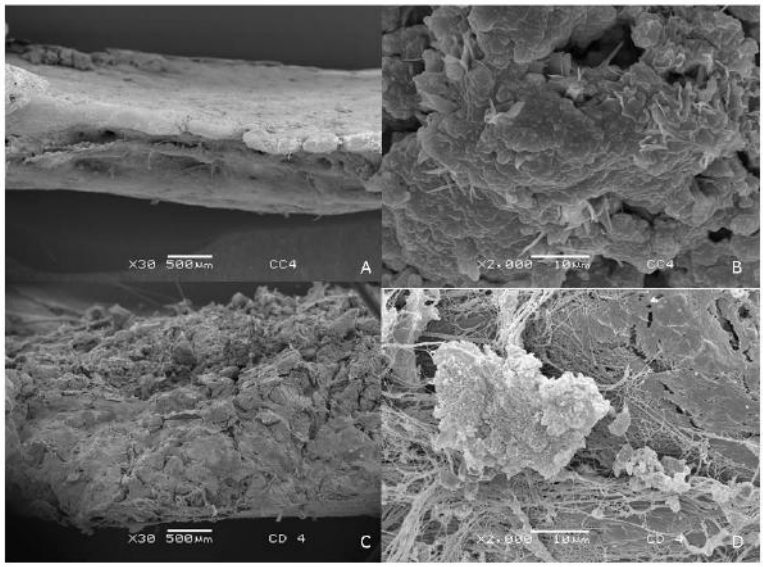


Source: Authors.

3.2 Group 2 – 8 weeks

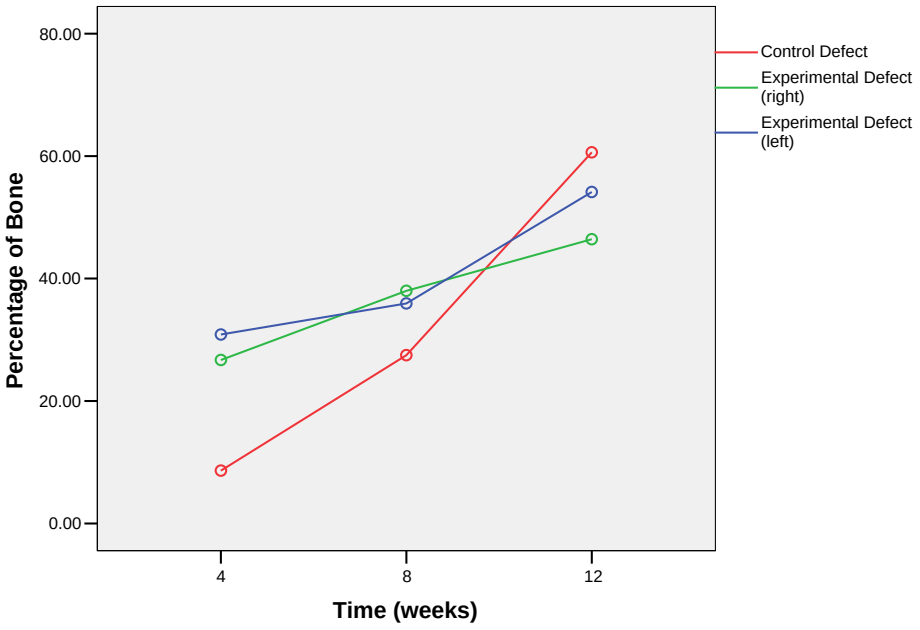
At this time, the amount of new bone formation in control and experimental defects was more expressive than that presented in the 4 weeks Group (Figure 2). In the control defects, the bone formation was more evident in the periosteum side and from the surrounding edge of it. In the experimental defects, it was seen a greater presence of bone tissue around of the hydroxyapatite particles, as also seen in fluorescence microscopy (Figure 3). It was observed the presence of blood vessels and a fibrous connective tissue denser than at 4 weeks. Furthermore, HA particles were less numerous than in Group 1, but more numerous than observed in Group 3. In the SEM evaluation, osteoblasts were more fixed in the HA particles, as well as it was also seen in defects of Group 3 (Figure 4).

Figure 3 - Scanning electron micrographs of the bony defects surfaces. Osteoblasts, collagens fibers and hydroxyapatite particles (HA). A and B Control site in 12 weeks. C and, E Experimental site in 12 weeks.



Source: Authors.

Figure 4 - Graph representing percentage of bone during the observation time in control and experimental defects of Groups 1, 2 and 3.



Source: Authors.

3.3 Group 3 – 12 weeks

The new bone formation in the control and experimental defects was greater in this period than observed at 4 and 8 weeks. The control sites exhibited bony trabeculae formation projecting toward the defect with an adjacent fibrous zone smaller than in Group 2. The new bone presented numerous and large medullar spaces. In the defects with bone graft, there was few and small HA particles, indicating a progressive absorption of the biomaterial, also observed in fluorescence microscopy and SEM (Figure 4). These particles were included in a new bone tissue and few amount of dense connective tissue. In fluorescence microscopy, the HA particles were involved by a large amount of bone, showed by the bony makers lines around them. The soft tissue between the graft particles and the new formed bone was denser than in the Groups of 4 and 8 weeks. At 12 weeks, it was verified more lamellar organization of the bony tissue, principally in the experimental defects, also evident in fluorescence analysis.

3.4 Histomorphometric Analysis

The histomorphometric analysis showed that the percentile of new bone formation in the experimental defects at 4 weeks period was statistically the same as that one in the control defects with 8 weeks (Table 2 and Figure 5). At 12 weeks, it was verified a higher percentile of bone in the control defects. However, the control and experimental defects at the left side of the midline, at 12 weeks, presented statistically the same quantity of connective tissue (Table 2, Figure 6).

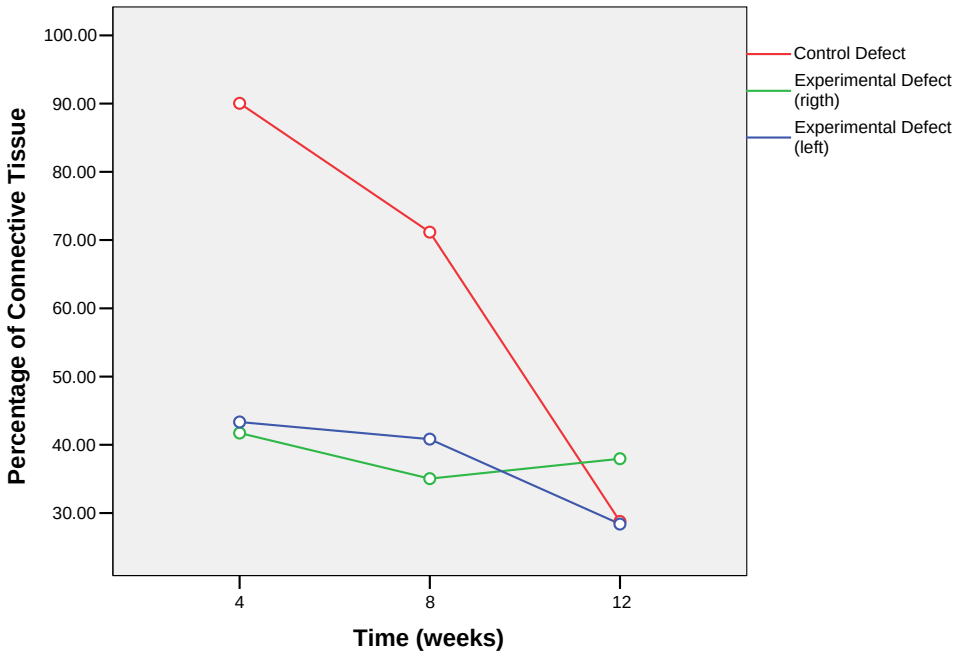
The analysis of the percentile of HA and the area occupied by its particles in the graft defects revealed that it had gradually decreased along the experimental period of 4, 8 and 12 weeks (Figure).

Table 2. Mean and (standard deviation) of bone, connective tissue and HA percentage in control and experimental defects. * There was not value to HA in control defects. CD – Control Defect; ED – Experimental Defect. p≤ 0,05.

GROUPS	BONE	CONNECTIVE TISSUE	HA
	F=247.804;df=8	F=175.000;df=8	F=137.722;df=8
CD- 4 weeks	8.63 (2.09)	90.05 (4.08)	0 *
ED (right) - 4 weeks	26.71 (2.06)	41.72 (4.16)	22.42 (3.20)
ED (left) - 4 weeks	30.87 (2.88)	43.34 (3.23)	19.42 (2.90)
CD - 8 weeks	27.49 (2.01)	71.16 (2.06)	0 *
ED (right) - 8 weeks	38 (4.74)	35.04 (7.43)	19.09 (4.27)
ED (left) - 8 weeks	35.94 (2.85)	40.83 (2.13)	16.86 (2.67)
CD - 12 weeks	60.61 (4.04)	28.77 (8.02)	0 *
ED (right) - 12 weeks	46.43 (3.34)	37.96 (5.35)	12.42 (2.73)
ED (left) - 12 weeks	54.14 (3.28)	28.38 (4.27)	10.33 (1.73)

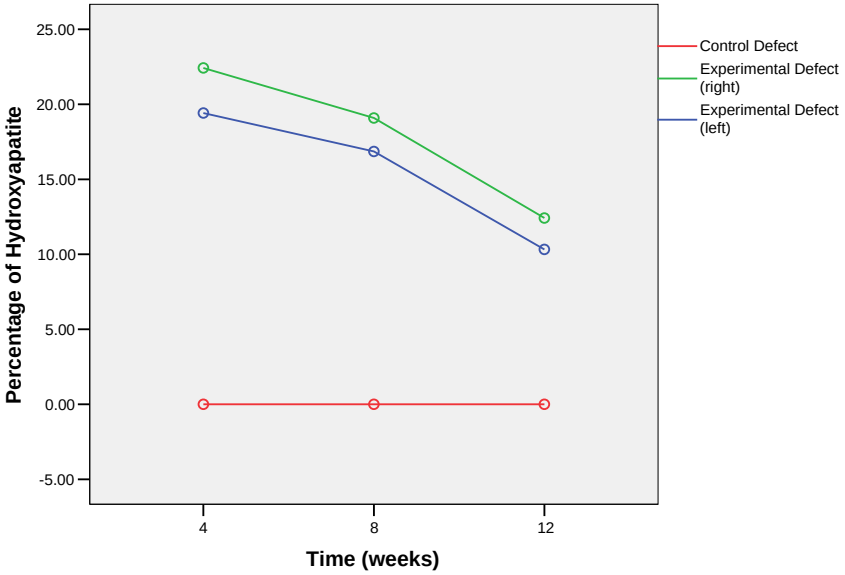
Source: Authors.

Figure 5- Graph representing the percentage of connective tissue during the observation time in control and experimental defects of Groups 1, 2 and 3.



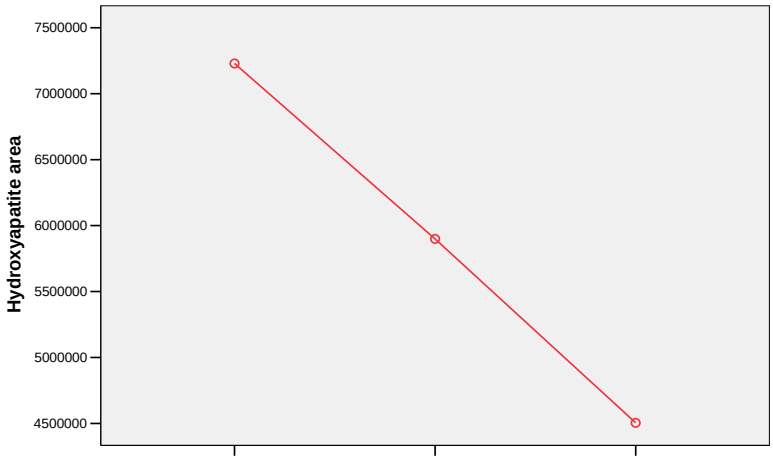
Source: Authors.

Figure 6 - Graph representing the percentage of hydroxyapatite during the observation time in control and experimental defects of Groups 1, 2 and 3.



Source: Authors.

Figure 7 - Graph representing the area occupied by the particles of HA during the observation time of 4, 8 and 12 weeks.



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4. Discussion

Autogenous graft, the gold standard material used in buco-maxillo-facial and orthopedic surgeries, presents high donor site morbidity and difficulty to be adjusted to the desired shape into the receiver site (Hsu, et al. 2005). Substitutes such as HA and other ceramics have shown variable results for this surgical purpose, but most of the authors agree that the HA composites satisfy many, if not all, requirements as an ideal biomaterial for bone substitute (Gosain, et al. 2005).

The graft composed of hydroxyapatite and collagen type I used for this study revealed biocompatibility due to absence of infections and postoperative complications during the experimental period as shown by other authors in the literature

(Gosain, et al. 2005, Harvey, et al. 1985, Holmes, et al. 1988, Jensen, et al. 2006, Klinge, et al. 1992, Mehlisch, 1989, 2005, Rezende, et al. 1996, Sculean, et al. 2005, Tachibana, et al. 2003, Thorwarth, et al. 2005). Bone tissue of varied extension had been formed in direct contact to the most of HA particles in all groups. In the Group of 12 weeks, the particles were smaller and in less amount than in Groups of 4 and 8 weeks, with more bone surrounding them. These findings were confirmed by the light and fluorescence microscopy analysis. Furthermore, some particles of HA appeared to be surrounded by connective tissue mainly at the early stages, like others studies had already shown (Araujo, et al. 2001, Cardaropoli, et al. 2005, Gosain, et al. 2005, Hallman, et al. 2001, Hsu, et al. 2005, Klinge, et al. 1992, Mehlisch, 1989, Minabe, et al. 1988, Silva, et al. 2005, Tachibana, et al. 2003). As opposed to these results, Hass et al. (1998) detected new bone formation around the HA only in the contact area of the receiver site bone surfaces (Haas, et al. 1998). The histological findings of the present study confirmed the osteoconductive property of the HA when placed in an osteogenic environment (Haas, et al. 1998, Hallman, et al. 2001, Lew, et al. 1997, Pettis, et al. 1990). However, the mechanism leading to the formation of new bone in direct apposition to the ceramic material as well as the composition of the chemical produced to bond the graft and bone remains unclear (Tachibana, et al. 2003). Some authors reported the osteoinduction property of HA-derived biomaterials after its subcutaneous implantation within different animal models (Gosain, et al. 2005, Gosain, et al. 2002, Ripamonti, 1996), but none conclusion about this could be drawn in the present research.

The hydroxyapatite graft used in this study was shown as an absorbable material as proved by the histomorphometric analysis of the area occupied by HA particles. It was smaller in the Group of 12 weeks as well as that was already related in the literature (Araujo, et al. 2001, Hsu, et al. 2005, Thorwarth, et al. 2005). Nevertheless, its process of degradation and elimination occurs in a slow gradual manner and not totally understood (Tachibana, et al. 2003). Probably, this phenomenon results from gradual dissolution of the HA particles in tissular fluid and the process of cellular mediation (Frame, 1987). Some authors consider that HA is absorbed and replaced by a new bone (Aichelmann-Reidy, 1998, Indovina, 2002, Lew, et al. 1997, Nishikawa, et al. 2005, Silva, et al. 2005). Furthermore, the calcium sulfate dissolved could act as a source of calcium ions that will be incorporated in the bone (Aichelmann-Reidy & Yukna 1998). However, other studies had not observed the HA potential of absorption (Hallman, et al. 2001, Holmes, et al. 1988, Jensen, et al. 2006), being this due to the kind of HA used in these experiments. Regarding the collagen, the other component of the graft material applied in this study, it was probably degraded by the collagenases enzymes and replaced by the new formed bony tissue (Hsu, et al. 2005, Ishikawa, et al. 2001).

One of the purposes of placing biomaterials in hard tissue defects is to offer stability for the coagulum and provide a scaffold for new bone formation (Cardaropoli, et al. 2005). This objective was found in the current study, mainly in the groups of 4 and 8 weeks, that had presented more bone formation in the experimental than in the control defect sites. Some authors had already concluded that a large amount of bone is formed in the early phase of the healing process, between 1 and 6 weeks, (Fujishiro, et al. 1997, Ishikawa, et al. 2001, Lew, et al. 1997), which is in agreement with the findings of this research. The greater presence of bone in the early stages of the experiment could have been a result, in part, of remodeling of the implant site attributed to Wofff's law phenomenon (Lew, et al. 1997).

Although it was detected a higher percentage of bone in the control sites, on 12 weeks' period, the amount of connective tissue was statistically the same between the control and experimental defects at the left side. It showed that the spaces not fulfilled with bone were being occupied by HA particles. One reason that justifies this can be the incompletely degradation of the HA, so the percentage of the connective tissue was the same at 12 weeks in the control and experimental sites. This soft tissue between the HA particles and bone seemed very dense and with osteogenic potential. Besides, the thickness of the experimental defects was visibly larger than the control ones, fact confirmed by SEM analysis of the current study and also already related in the literature (Gosain, et al. 2005). The maintenance of the volume is a desirable property of the graft materials, mainly for alveolar ridge augmentation (Araujo, et al. 2002, Deeb & Roszkowski, 1988, 1989, Taylor &

Helfrick, 1989).

The amount of new bone formation was smaller in the central area of the defects than in its periphery in all samples, which is according to another study in the literature (Fujishiro, et al. 1997). The rates of bone formation increased in the course of time while the areas of soft tissue and HA had decreased.

The association of HA and collagen is recommended by some authors to improve the properties of graft materials. It can maintain the particles in the surgical place for more time (Carvalho, 2000, Deeb & Roszkowski, 1988, Hallman, et al. 2001, Hsu, et al. 2005, Mehlisch, 1989), as well as, it contributes to mineral deposition, vascular ingrowth and growth factor binding (Giannoudis, et al. 2005). This kind of graft can also act as a cell adhesive, promoting aggregation of fibroblasts and inducing osteoblasts proliferation, which should accelerate bone regeneration (Hsu, et al. 2005, Ishikawa, et al. 2001, Li, H., Zou, X., et al. 2005). Furthermore, the collagen affects migration and differentiation of mesenchymal cells, provides the initial precipitation and the support for the newly formed mineral crystals (Li, et al. 2005). Minabe et al. (1988) suggested that the complex of HA and collagen promoted more new bone formation than the HA isolated in periodontal osseous defects (Minabe, et al. 1988). All of these reasons already available in the current literature contributed for the selection of a graft with 75% of HA associated to 25% of collagen type I, in order to perform the present study. Optimal chemical and physical properties of this biomaterial were expressed by the clinical and histological results obtained during the healing phase and new bone formation process in the experimental period.

5. Conclusion

The biocomposite of hydroxyapatite and collagen type I showed biocompatibility with a tenuous amount of inflammatory cells, but accelerated new bone formation at early stages of healing. The graft particles were slowly absorbed at the same time while the bone was being formed. More studies are necessary to prove the absolute absorption and its total replacement by new bone, but it can be concluded that this material is an excellent bone graft in reconstructions of bone defects.

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