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Initial endosseous healing response to lactide/glycolide copolymer

Estudo da resposta tecidual inicial em defeitos ósseos enxertados com PLGA

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Keywords:
bone graft; synthetic biomaterial; PLGA.

Abstract

Introduction: The search for less invasive treatments, with fast and effective bone regeneration, has lead to the development of synthetic bioresorbable alternatives for bone graft. The commercial product Fisiograft™ gel (Ghimas Spa, Italy), based on lactide-glycolide copolymer, is used as an injectable biocompatible and bioresorbable material for filling bone defects in dental surgery applications. These polymers are also used in the production of suture threads, pins and plates for bone fixation, and barriers for guided tissue regeneration. **Objective:** The main objective of this pilot study was to observe the initial bone healing response after the application of polylactide-glycolide graft material in adult animals. **Material and methods:** Bone defects were prepared in right and left femora of 12 month-old male Wistar rats. The defects in one leg received the synthetic graft material and the contra-lateral defects did not receive any treatment. After four days, the

animals were subjected to euthanasia, the femora were removed, and tissue blocks were prepared for histological analysis. **Results:** Blood clot remained in the centre of control defects with initial connective tissue organization on the edges. The graft material was observed in the centre of the treated defects, restricted to the area where it was applied, with neovascularization next to the graft material. The pattern of bone healing did not differ between groups, starting from the margins of the defects and from bone fragments, with neovascularization followed by deposition of non-mineralized bone matrix towards the centre. **Conclusion:** The results indicate that the lactide-glycolide copolymer gel was effective as a filling and osteoconductive material, allowing tissue healing during its resorption process. Additional studies are necessary to verify its capacity to promote bone regeneration.

Resumo

Palavras-chave:
enxerto ósseo;
biomaterial aloplástico;
PLGA.

Introdução: A busca de tratamentos menos invasivos, com rápida e efetiva regeneração óssea, tem levado ao desenvolvimento de alternativas sintéticas e biorreabsorvíveis para enxertos ósseos. O material sintético à base de copolímeros de ácidos polilático e poliglicólico (PLGA), no produto comercial Fisiograft™ gel (Ghimas Spa, Itália), é indicado pelo fabricante como dispositivo biorreabsorvível e biocompatível para preenchimento de defeitos ósseos em periodontia, cirurgia bucomaxilofacial e implantodontia. Esses polímeros são usados também na fabricação de fios de sutura, pinos e placas para fixação óssea em ortopedia e barreiras para regeneração tecidual. **Objetivo:** Este estudo piloto in vivo teve como objetivo principal observar o período inicial da resposta óssea na aplicação de PLGA em animais adultos. **Material e métodos:** Foram produzidos defeitos ósseos em cada fêmur de ratos machos Wistar com 12 meses de idade. Metade dos defeitos foi preenchido com Fisiograft™ gel, e defeitos contralaterais não receberam material de preenchimento. Os animais foram submetidos a eutanásia após quatro dias do pós-operatório, e os blocos de tecido foram preparados para análise histológica. **Resultados:** O centro do defeito no grupo controle encontrou-se preenchido com coágulo sanguíneo, enquanto o tecido conjuntivo se organizou próximo às bordas. Nos defeitos enxertados foi verificado que o gel se manteve restrito ao local em que foi aplicado, com neovascularização próxima ao copolímero. O padrão de deposição óssea não diferiu entre os grupos, sempre a partir das margens do defeito e da superfície dos fragmentos ósseos, representado pela deposição de matriz não mineralizada em direção ao centro do defeito. **Conclusão:** Neste trabalho, a utilização do gel mostrou-se eficaz como material de preenchimento e osteocondutor, possibilitando neovascularização e reparo tecidual inicial durante sua reabsorção. Estudos adicionais são necessários para verificar o efeito desse biomaterial em períodos maiores e para esclarecer a possibilidade de o material promover a regeneração óssea.

Introduction

The clinical application of synthetic biomaterials in medicine and dentistry is evolving rapidly due to their bioresorbable potential. Biocompatible alternatives, less invasive than autografts, are being developed for filling bone defects, guiding tissue regeneration and as carriers for osteogenic cells and induction factors [13, 15, 25].

Materials based on polylactide (PLA), polyglycolide (PGA) and their copolymers (PLGA) can be manufactured in different proportions, molecular weights, densities, morphologies etc. The degradation rate of this copolymer can be controlled by the proportion of PLA:PGA, because PLA is less susceptible to degradation for being more stable, with greater half-life time in vivo [29]. Devices based on PLGA, such as pins, screws, plates and membranes, were extensively studied and used in orthopedic surgeries [2, 7, 17, 22]. Since the mechanical, physical and chemical properties of a material determine the tissue response towards it, many studies modulate these properties to develop the material most indicated for specific requirements, including tissue engineering [10, 11, 13, 14, 19, 23]. The association with other polymers, ceramics and bioactive components increase even more their possibilities of use.

According to Vert et al. [29], the prerequisites for the clinical application of synthetic polymers are: biocompatibility, biofunctionality, stability and bioresorbability. The bioresorption process common to PLGA, resulting in total elimination of the material, comprises an in vivo degradation by a hydrolytic reaction, resulting lactic and glycolic acids, eliminated through physiological mechanisms.

Possible discrepancies on the tissue response observed towards the PLGA were explained by the differences in morphology and compositions, in addition to the presence of solvents, sterilization or storage alterations, radiation and adsorption of water [29].

The mild inflammatory response described in more recent studies was observed only in initial wound healing periods, with the presence of macrophages and multinucleated giant cells [2, 15, 18], without interfering with bone matrix deposition. PLGA discs promoted higher bone matrix deposition in critical size defects when compared to controls, without chronic inflammatory response and debris, indicating biotolerance towards it [16]. PLGA scaffolds used as aloplastic graft yielded a greater bone formation index in critical size defects, and the association with osteogenic cells indicated a faster healing at early time points [6]. The association of PLGA with cells [6], proteins [16] and growth factors [31] presented a synergistic effect on bone healing, with a very promising use in bone engineering.

The commercial product Fisiograft™ (Ghimas Spa, Italy), based on PLGA, is supplied as sponge, powder and gel. It is indicated for maxillofacial surgeries, as a biocompatible and absorbable "space maintainer". The manufacturer suggests that the lower density of this material allows a gradual bone healing process while it is being resorbed. Carvalho et al. [3] indicated its use in sinus augmentation and implant fenestration associated to autogenous particulate bone.

Serino et al. [26] observed that the vertical alveolar resorption, frequently following teeth extraction, was prevented or reduced in Fisiograft™ sponge grafted sockets. This ridge preservation technique formed a barrier and prevented the collapse of soft tissue during bone healing.

In six months, the newly deposited bone matrix presented remodeling signs, without debris of the graft material, showing to be ideal for dental implant placement. The grafting of periodontal intra-osseous defects with Fisiograft™ was evaluated by Minenna et al. [20] and Stratul et al. [27], who concluded that there was not a significant improvement in probing depth and clinical attachment levels after this reconstructive surgery, compared to open flap and debridement procedures.

There are a growing number of PLGA materials available for filling bony cavities and alveolar sockets, reconstructive surgery and barriers for guided tissue regeneration. The understanding of the tissue response towards the graft materials, presented in different polymer proportions and densities, is essential to investigate their bioresorption and effect on osteogenic processes. The aim of the study reported herein was to examine the initial bone healing response after the application of polylactide-glycolide gel graft material in adult animals.

Material and methods

Five 12 month-old male Wistar rats (325-350g) entered this pilot study, after the approval of this project by the Ethics Committee of Positivo University (number 159.05). The anaesthesia was induced with intramuscular administration of ketamine hydrocloride (Dopalen, Vetbrands, Brasil – 100 mg/Kg) with xylazine (Anasedan, Vetbrands, Brasil – 10 mg/Kg).

The surgical procedure was performed with the animals in the lateral decubitus position, onto dry paper padding after shaving and preparing the hind limbs with 10% povidone-iodine antiseptic solution (Iodofor, Multilab, Brasil). Each femur was exposed through a lateral longitudinal incision

extending between the vastus lateralis and adductor muscles. The distal metaphysis was exposed and the periosteum was stripped from the bone. Bone defects were prepared in the right and left femora of each animal, at approximately 6 mm from the knee in order to avoid interference with the growth plate. A circular opening was created perpendicular to the lateral cortex with a 2.0 mm diameter carbide bur (nº 56, Maillefer, Brasil; Dentec Motor 405N, Brasil), under saline irrigation.

The defects were finalized when the bur touched the endosteum of medial cortex, with saline solution irrigation. One defect of each animal was filled with Fisiograft™ gel to the external cortical level, while the contra-lateral femur did not receive any treatment. The incision was closed in two layers with silk thread 3.0 (Johnson's, Brasil). Acetylpromazine (Profenid, Aventis, Brasil – 2 mg/Kg) was administered intramuscularly and Ibuprofen (Spidufen 400 mg, Zambon, Brasil) was in water ad libitum.

In the fourth postoperative day the animals were euthanized in CO_2 gas chamber, the femora were dissected and the tissue blocks were left in 10% buffered formalin solution for at least 48 hours. Fixed samples were decalcified in 10% formic acid for 48 hours, dehydrated in graded ethanol and embedded in low melting-point paraffin (Biotec, Labmaster, Brasil). The blocks were sectioned perpendicularly to the defects and the sections were stained with either Masson trichrome or Giemsa for light microscopy histological observations (Olympus BX41, USA).

Results

It was observed that the defects in the control group were filled with blood clot in the centre and connective tissue around the edges. Areas with edema, low cell density, fibers and neovascularization were observed (figure 1). Bone matrix deposition was limited to the surface of pre-existing bone (figure 2).

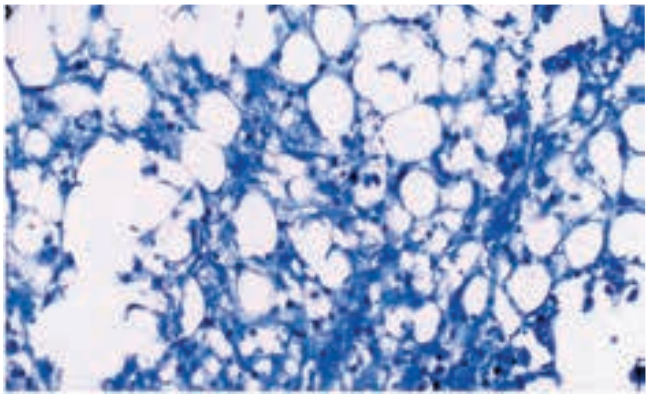

Figure 1 — Blood clot at the centre of the control group defect (Giemsa, 100X)

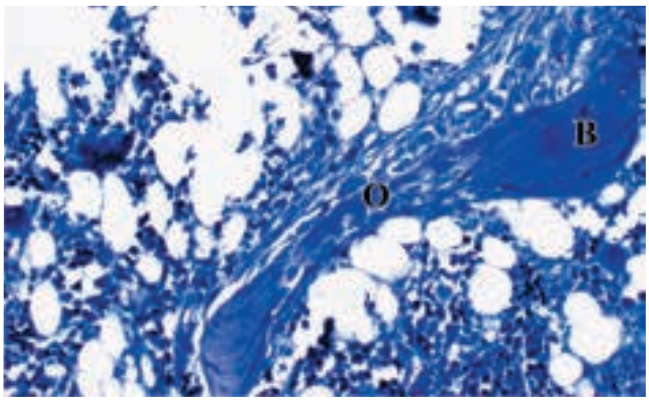

Figure 2 — Bone matrix characterized by Osteoid (O) deposition from bone fragments (B) in the control group (Giemsa, 100X)

Remnants of the PLGA gel graft material filled the centre of the grafted defects and were restricted to this area (figure 3). Copolymer agglomerates, with blood cells embedded, presented two distinct morphologic aspects, fibrillar or globular (figure 4).

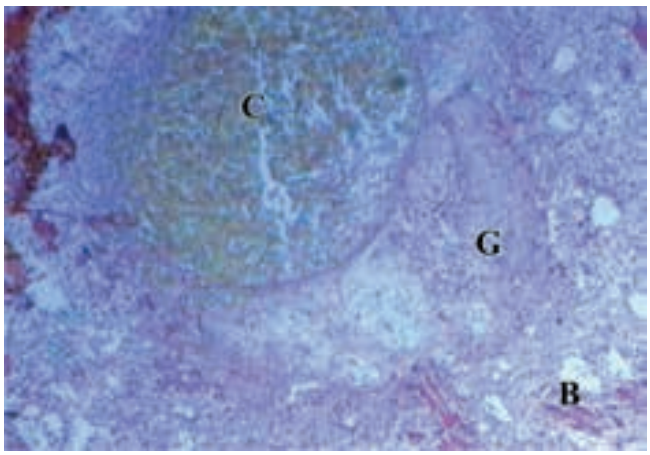

Figure 3 — Centre of the defect grafted with Fisiograft™ gel: clot (C), gel (G) and bone fragments (B) (Masson trichrome, 40X)

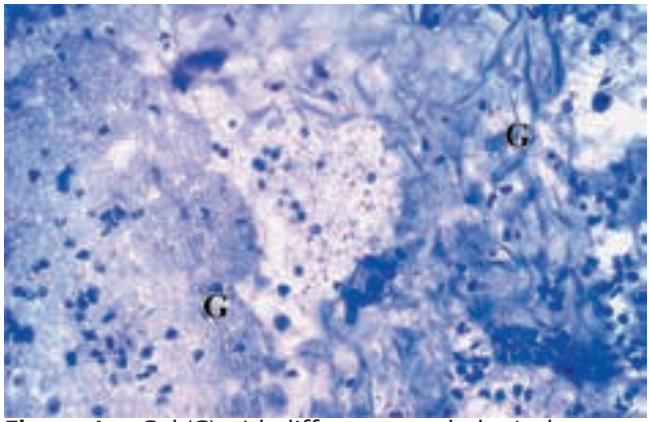

Figure 4 — Gel (G) with different morphological aspects (Giemsa, 100X)

The highly vascularized connective tissue, which precedes bone deposition, was observed between graft material and the existing bone and between the gel agglomerates (figure 5). Macrophages were sporadically observed around the graft in one of the defects (figure 6).

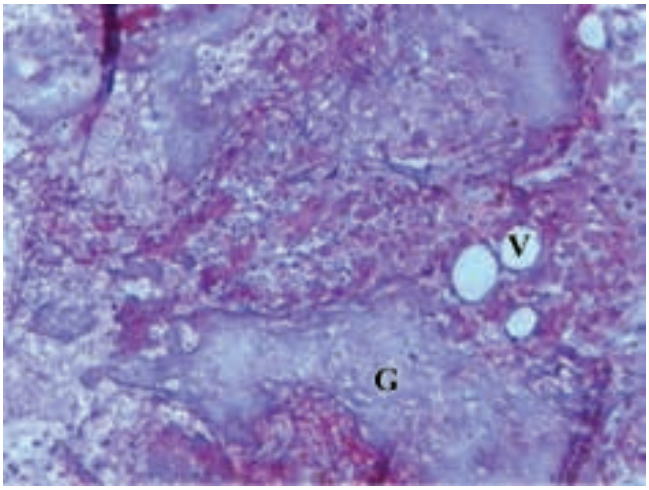

Figure 5 — Granulation tissue next to the gel (G), with neovascularization (V) between agglomerates of the material (Masson trichrome, 100X)

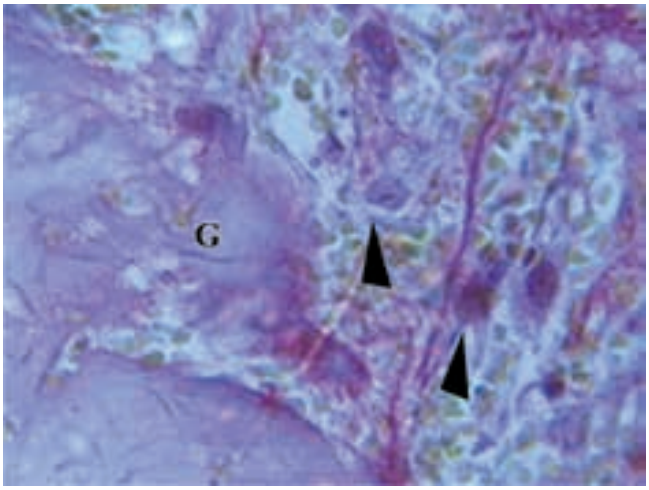

Figure 6 — Macrophages (arrow head) around the material (G) (Masson trichrome, 400X)

There was no difference on bone healing patterns between control and grafted groups. Bone deposition from the surfaces covered with osteogenic cells was characterized by a non-mineralized osteoid matrix (figures 7 and 8).

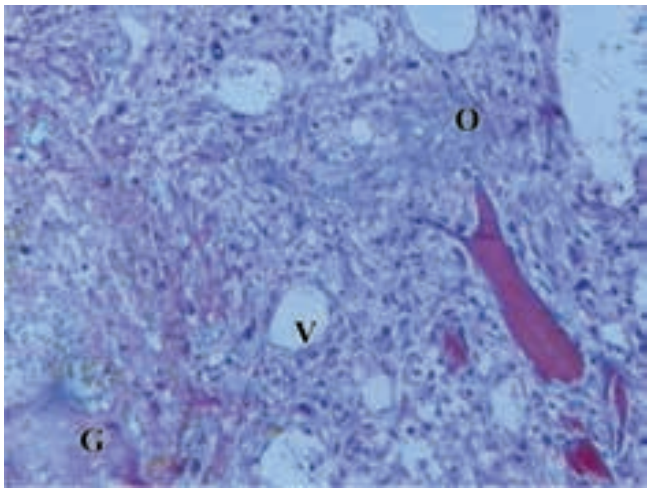

Figure 7 – Unmineralized bone matrix deposition (O) from a bone fragment next to the gel (G), in a highly vascularized area (V) (Masson trichrome, 100X)

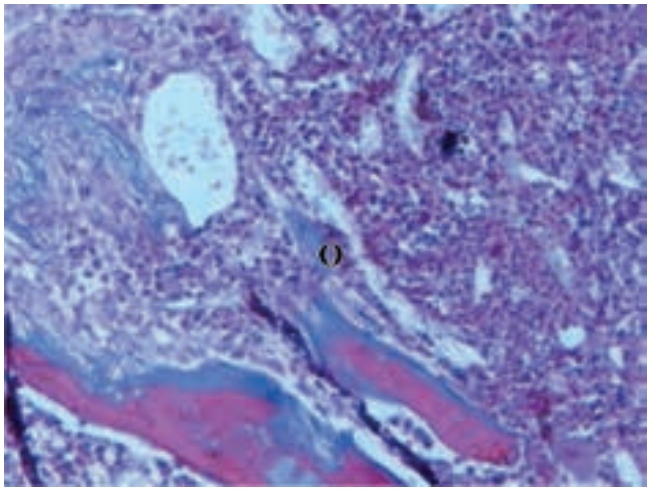

Figure 8 – Deposition of bone trabeculae (O) from trabecular bone in the grafted group (Masson trichrome, 100X)

Discussion

The creation of a mechanically stable bone defect is followed by blood loss, clot formation, development of temporary vascularized tissue and ingress of osteogenic cells and deposition of a trabecular bone matrix. Endothelial and osteogenic cells migrate through the fibrin scaffold of the blood clot. Bone deposition requires more time and more differentiated cells than fibroblasts, present at initial healing time in bone repair. Clot resolution, when accompanied by fibrous tissue development and contraction of the tissues that precede bone deposition, characterizes the non-healing structure

of critical-size defects or loss of bone dimension, so common in dental extraction.

The use of the terminology "bone regeneration" can be controversial. Davies and Hosseini [4] consider that the complete reconstitution of a tissue to the organization before the lesion is a unique feature observed only in bone, while the other tissues, except embryogenic, are repaired with the formation of a fibrous tissue.

The structural differences between the bone matrices deposited initially and during remodeling are also very important, bearing in mind that the primary trabecular matrix has a much higher deposition rate. Therefore, a material that fills a bone defect and is gradually resorbed concomitantly with bone deposition presents the advantage of maintaining the space volume during the period necessary for the effective bone regeneration. During the bioresorption of the graft material, the defect must be progressively filled by trabecular bone [9].

The Fisiograft™ gel (100 mg 50:50 PLA and PGA, in 400 mg polyethylene glycol) is considered a biocompatible material [29], even though it does not correspond to the classic definition of the term, because it integrates with the biological system during its local bioresorption process and bone deposition.

The expansion of products with permanent applications, thus biostables, seeks minimal interaction between material and tissue. On the other hand, for bioresorbable materials the situation is the opposite, because the material is a source of products that interact with the system.

In Williams' opinion [30], the biocompatibility concept should be revised in order to recognize that the biological response towards a bioresorbable material could be beneficial if adjusted, in the development of a material, to take advantage of this interaction. Anderson [1] considers that the development of granulation tissue and foreign-body response, part of the normal interaction tissue and resorbable material, needs to be differentiated from the chronic inflammatory process.

The presence of macrophages observed at four days around the Fisiograft™ gel could be related to this healing period, indicating the initial process of bioresorption. Due to the macrophage incidence in immunological, anti-inflammatory and antimicrobiological responses, their occurrence in the healing response to an implanted material might be precipitously considered negative for tissue healing.

Macrophages are considered responsible for the response to bioresorbable materials, participating in the biodegradation via fagocitosis and extracellular degradation, organizing the neoangiogenesis and regulating the tissue regeneration [1, 32]. In a morphological variation of this response, in the presence of particles larger than the phagocytic capacity of macrophages, multinucleated cells or foreign bodies are formed by the fusion of monocytes and macrophages [1].

Giant multinucleated cells were observed on the surface of various structures denser than Fisiograft™ gel, but also based on PLGA implanted in bone, like pins [22], discs [18], and porous scaffolds, without interfering with bone deposition inside the pores [15].

Athanasiou et al. [2] and Vert et al. [29] noted that the inflammatory response observed towards the PLA and PGA is temporary and limited to the areas adjacent to the materials. During healing processes of repair and regeneration, cells and tissues are modulated and remodeled, and the bioresorbable material also goes through a continuous modification during the period it is implanted.

Vert et al. [29] reported that the initial stage of response to PLA and PGA, even in the presence of macrophages, multinucleated cells and fibroblasts, could evolve to two distinct situations depending on the chemical composition and structural configuration: materials with faster degradation are progressively substituted by bone while materials with longer degradation could be covered by a layer of fibrous connective tissue.

The animals used in this study, 12 month-old rats, are considered mature and the animal age can increase the inflammatory response to bioresorbable materials as demonstrated by Athanasiou et al. [2]. In this circumstance, the deposition of bone can also be delayed by the resolution of the inflammatory process.

The lower osteoinductive capacity with aging was observed by Nishimoto et al. [21], with later chondrogenesis and osteogenesis, besides lower quantities of cartilage and bone. Torricelli et al. [28], comparing the bone regeneration of defects in femora of 13 and 18 month-old rats observed that the volume of matrix deposited was reduced in older animals but matrix maturation was not affected.

The choice of the experimental model is particularly important, considering that the regeneration or not of bone defects can be dependent on the age of the animal [8]; consequently, the higher or lower tolerance to biomaterials and the inflammatory response can depend directly on the model employed.

In the time period observed in this study, PLGA gel filled half of the defects, sustaining the blood clot and fragments of bone, allowing the organization of the healing tissue around it. In the control group, clot and connective tissue were less dense, with more space between cells and fibers. The higher tissue support provided by the graft material was also correlated with the integrity of the histological sections of this group. The highly vascularized connective tissue, organized primarily, directed the appositional matrix deposition towards the centre of the defect, from the endosteal surface of the margins and from the surface of bone fragments. Concluding, the graft material permitted the development of an extensive vascular net besides stabilizing clot and bone fragments, extremely determinant factors for bone healing.

The anchorage of structural proteins that precede bone deposition, beginning with the clot fibrin until the temporary connective tissue, support and orient the migration of osteogenic cells and the deposition of bone trabeculae, determining the osteoconduction [5]. The osteoconductive materials provide a three-dimensional structure, or scaffold for vascular development and bone deposition. The osteoconductive property of a bioresorbable material is related to the dimensional stability of the graft material, for a slow bioresorption can support neovascularization and anchorage of the osteogenic matrix. A fast bioresorption would cause the loss of the scaffold, which supports the matrix that precedes bone deposition, and the absence of the osteoconduction. The development of bioresorbable materials with an architecture that supports osteoconduction relies on a slow resorption concomitant with bone deposition [9].

Meikle et al. [18] suggested that the osteoconductive capacity of a material depends on the porosity during fabrication or created on it. Compact materials can form spaces during the degradation and resorption of a component at a faster rate, forming the porous scaffold necessary for osteoconduction. Rimondini et al. [24] studied bone regeneration in the presence of PLGA powder on polyethylene glycol and dextrin, forming a gel suitable for injecting in small osseous defects. The rapid resorption of the hydrosoluble matrix produced a porous structure of PLGA, which was able to promote the regeneration of critical bone defects in rabbit femora, without inflammatory infiltration in the only observation period of 30 days.

Even when particles of the aloplastic material are detected after the healing period, bringing about some reservations on their use, it is observed a significant improvement when compared to non-grafted models and at later observation periods [29]. It can be assumed that the bioresorption of a material might have a mutual influence on bone regeneration, related to systemic, local and material aspects.

Zaffe et al. [33] reported that a probable absorption of glycoproteins from body fluid determined the positive coloration of degrading Fisiograft™ gel, compared to non-colored undegraded material. Besides the different morphological aspects observed on the gel in this study, probably due to the biodegradation rate, the absorption of proteins with the evolution of the regenerative process must also influence the response towards the graft material.

In the case of bioresorbable materials, the adsorption and absorption of local proteins and biological fluids associated to their degradation rate and resorption of the products can orchestrate the biological response. The superior biocompatibility of the PLGA gel with time, resulting in complete biotolerance and absence of inflammatory response, could be investigated from histological observations at a sequence of time periods.

The development of connective tissue in the grated areas and the vascularization adjacent to the material confirm that it provided a biologically functional site for posterior bone deposition, even though there was not a suitable surface for osteoblastic cell organization, given the short period of observation in this study and the bioresorption of the material.

In an ultrastructural analysis of defects after 30 days grafted with Fisiograft™ sponge, powder, and gel, Imbronito et al. [12] observed bone matrix juxtaposed with the polymer and deposition of collagen directly onto it by fibroblast-like cells. Observations from biopsies of sinus lifting with Fisiograft™ gel, associated or not to Bio-Oss™ [33], revealed that the early matrix deposited on the connective tissue organized next to the gel reached polymer surface and the end of this process was marked by the transformation of osteoblasts into quiescent cells. Studies monitoring the long term morphological alterations and absorption of proteins and fluids can elucidate the osteopromotive capacity of the PLGA copolymers, suggested by Zaffe et al. [33].

The differentiation of osteoblastic cells and the matrix deposition in close proximity to the polymer confirm the osteoconductive capacity of the material and suggest a regeneration of the defect when it is completely bioresorbed. The continuous bioresorption of the gel, creating smaller polymer agglomerates with larger spaces for

neovascularization and bone deposition characterizes the polymer capacity to maintain the space orienting the healing process.

The bone healing pattern did not reveal significant differences between the groups, and non-mineralized matrix was observed only adjacent to surfaces previously covered with osteogenic cells. Even though the healing period of 4 days was insufficient to observe a mineralized matrix, the tissue response was more pronounced from the margins of the defect. Further study is recommended to analyse the response at various time periods, following the process of bioresorption of this material until bone regeneration. The present study corroborates others, which conclude that PLGA gel is bioresorbable, biocompatible and osteoconductor. It is recommended that the graft area be filled, sustaining the blood clot, stabilizing other particulate material, acting as a barrier to fibrous tissue and guiding the bone regeneration without the need to be removed later.

Conclusion

The polylactide-glycolide gel was effective as a filling material. In the only period of observation, the material did not induce an inflammatory response. Bioresorption concomitant with neovascularization and bone matrix deposition demonstrated the biocompatibility of the material. The osteoconductive function of the gel was substantiated by its ability to maintain the clot and orient the matrix deposition without altering the healing pattern. Additional studies, with rigorous control of the variables discussed previously, are necessary to verify the histodynamical alterations caused by the use of this material in longer time periods and also to prove its capacity to promote bone regeneration.

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