Characterization and biological evaluation of the introduction of PLGA into Biosilicato.

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Resumo:
The healing of large dimension bone defects and fractures due to traumas, tumors or congenital diseases are a great challenge to the medical field. In this context, biomaterials have been arising as an alternative to be used as a graft to bone repair. One of the most common materials used for bone tissue engineering are bioactive glasses and glass ceramics, such as Bioglass® and Biosilicate® (BS), respectively, which are a group of synthetic silica-based bioactive materials with the unique ability to bond to living bone. They are capable of forming a biologically active bone-like apatite layer on their surface that acts as a template for calcium phosphate precipitation and directs new bone formation. In addition, it has been suggested that BS attract and stimulate osteoprogenitor cells, which differentiate into matrix-producing osteoblasts and subsequently increase the rate of bone formation and bone ingrowth into BS-based granular material. They represent a promising candidate material for bone substitution mainly due to their biocompatibility and osteoconductive properties. An additional advantage of such material is the self-setting nature, which makes them injectable and allows the use of a minimally invasive surgical procedure during clinical use. However, BSs are characterized by a very slow degradation rate, which has to be considered as a disadvantage for several applications. To enhance the biodegradability and tissue ingrowth of BS, (micro)porosity can be introduced into the already intrinsically nanoporous cement. One promising strategy to create porosity into BS is the introduction of biodegradable polymers, e.g. in the form of poly(D,L-lactic-co-glycolic) acid (PLGA) microparticles. However, the biological effects of BG and PLGA composites were not studied yet. Thus, the aims of this study were to characterize different BS/PLGA composites for their physicochemical and morphological characteristics and evaluate the in vitro and in vivo biological performance of the composites. Biosilicate® (BS) parent glass was used as the bioactive glass (quaternary P2O5-Na2O-CaO-SiO2 system. For the preparation of PLGA microspheres, poly(lactic-co-glycolic) acid (PLGA; Purasorb® 5002A, Purac, Gorinchem, The Netherlands) was used. PLGA microspheres were prepared according to a previously described single emulsion technique. For this study, four different groups were used: BS; BS/PLGA-80/20; BS/PLGA-70/30 and BS/PLGA-60/40, with the numbers representing the weight ratio of BS and PLGA. BS and PLGA microspheres were added in a syringe with 2% Na2HPO4 and mixed for 20 s, using a mixing apparatus (Silamat® S6, Ivoclar Vivadent, Amherst, USA). Immediately after mixing, the composites were injected into Teflon molds (8 mm in diameter and 2 mm thick for in vitro tests and 3 mm in diameter and 2 mm thick for in vivo tests). After overnight setting at room temperature, the composites were removed from the molds and sterilized by ethylene oxide (Acecil, Campinas, SP, Brazil). For this study, four different groups were used: BS; BS/PLGA-80/20; BS/PLGA-70/30 and BS/PLGA-60/40, with the numbers representing the weight ratio of BS and PLGA. The physicochemical and morphological modifications were analyzed by difração de raio x (RXD), setting time, pH, mass loss and SEM. For in vitro analysis the osteoblast and fibroblast viability was evaluated after 24, 48 and 72 hours after seeding. Histology and immunohistochemistry were performed in the tibial bone defect model in rats to evaluate the in vivo response. XRD analysis and SEM images confirmed that the composites degraded over time. Additionally, pore formation was observed after incubation mainly in BS/PLGA groups. BS setting time was significantly higher compared to composite groups. Moreover, after incubation in PBS, all composites presented lower values for pH and mass loss over time. BS/PLGA showed significantly increased in osteoblast viability 24 hours after seeding. Moreover, BS/PLGA composites demonstrated an increase in fibroblast viability 24, 48 and 72 hours after seeding when compared to BS. In the in vivo experiment, after 2 and 6 weeks of
implantation of biomaterials, histopathological findings revealed that the BS/PLGA composites degrades over time, mainly at periphery. Moreover, histological sections demonstrated the presence of granulation tissue and bone formation in all groups analyzed. Immunohistochemistry analysis demonstrated that BS and BS/PLGA composites stimulated Runx-2 and RANKL. In conclusion, the results of this study showed that BS/PLGA composites present appropriate physicochemical characteristics at all formulations. Also, BS/PLGA composites are biocompatible and presented a faster material degradation rate, allowing replacement by bone ingrowth.