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GELATIN-BASED BIOMATERIAL FOR PERIODONTAL REGENERATION

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1 INTRODUCTION

Periodontal disease (PD), caused by plaque and an exaggerated inflammatory response, represents a problem in veterinary dental care. In dogs, poor oral hygiene and cavity size lead to rapid progression from gingivitis to severe periodontitis, causing tissue destruction and teeth loss due to loss of attachment [1]. The goal in regenerating lost periodontal tissues is to achieve the well-being of the animal and restoration of normal function and structure, yet it remains a significant challenge due to the complex nature of the disease [2]. Guided tissue regeneration (GTR) is a periodontal therapy used after administration of antibiotics and dental scaling to help delay the disease progression and allow regeneration of alveolar bone [3]. The use of membranes creates a barrier between the defect and the epithelial tissues allowing the regeneration of the lost tissues while maintaining the passage of nutrients [4]. Our study focused on the production of a customizable gelatine-based hydrogel with osteoconductive properties with potential to be used in the manufacture of membranes for periodontal regeneration.

2 METHODOLOGY

2.1 GELATIN-BASED HYDROGEL PRODUCTION AND CHARACTERIZATION

Gelatin-based hydrogels with graphene oxide (GO) and hydroxyapatite nanoparticles (HAp NPs) were fabricated by a simple crosslinked hydrogel technique. Composite hydrogels were maintained at 75 ºC for 1 hour in a water bath to allow proper crosslinking and ensure a homogenous structure. The hydrogels were characterized using ATR-FTIR spectroscopy to identify the functional groups and scanning electron microscopy (SEM) to examine their morphology, distribution and size.

2.2 CYTOCOMPATIBILITY AND OSTEOGENIC POTENTIAL OF THE HYDROGELS

To assess *in vitro* cytocompatibility and osteogenic potential of the gelatin (Gel)-based hydrogels, sterilized composite hydrogels (Gel, Gel_HAp, Gel_GO, Gel_HAp_GO) were incubated in basal medium with antibiotics (37º C, 5% CO_2/air) for 24 hours to obtain extracts (0.1 g/mL, according to the norm ISO 10993-12:2004). Canine periodontal ligament (PDL)-derived cells were obtained by the explant technique [5] and cultured in basal medium (37º C, 5% CO_2/air) until 80% confluence was achieved. PDL-derived cells passage 3 were cultured for 48 hours to adhere and, after, extracts at 5% and 10% were added to the cultures. Cells cultured in the absence of the gels were used as control. Cultures were grown for up to 14 days after the addition of the extracts and characterized for metabolic activity, morphology and osteogenic markers. Comparison of the conditions was assessed using the t-test and by the one-way analysis of variance, followed by the post hoc Tukey.

3 RESULTS AND CONCLUSION

The incorporation of GO and HAp nanoparticles enriched the gelatin-based hydrogels with osteoconductive properties while preserving the structural integrity of both nanoparticles. The addition of GO significantly increased the elastic behavior of the gelatin-based hydrogel. Control cultures exhibited metabolic activity that increased over the culture time. Test conditions (Gel_HAp, Gel_GO, Gel_HAp_GO hydrogels) also showed increasing metabolic activity and cell proliferation, with characteristic fibroblastic morphology. Alkaline phosphatase (ALP) levels, an osteogenic marker, increased throughout the culture time. Collagen and ALP presence were confirmed by staining.

In conclusion, the addition of HAp NPs and GO to the hydrogel did not compromise the structural integrity and enhanced the proliferation of dog PDL-derived cells, suggesting its potential to be used in the manufacture of new regenerative GTR techniques for the control of tissue destruction in dog's periodontal disease.

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