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Feasibility study of collagen membranes derived from bovine pericardium and intestinal serosa for the repair of cranial defects in ovariectomised rats

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ABSTRACT

The indication of biomaterials has increased substantially in the regenerative therapy of bone defects. However, in addition to evaluating the physicochemical properties of biomaterials, the quality of the recipient tissue is also essential for the osseointegration of implants, as abnormalities in bone metabolism, such as gonadal hormone deficiency, can influence bone healing. This study evaluated the osteoregenerative capacity of collagen membranes derived from bovine pericardium and intestinal serosa in the repair of cranial defects in ovariectomised rats. Thirty female Wistar rats were submitted to surgical creation of a 5-mm cranial bone defect. The rats were divided into a control group (not ovariectomised) and an ovariectomised group. The non-ovariectomised group was divided into three subgroups: control (G1) in which the defect was not filled with the biomaterial, and two subgroups (G2 and G3) that received the bovine pericardium- and serosa-derived collagen membranes, respectively. The ovariectomised group was divided into the same subgroups (G4, G5, and G6). The animals were sacrificed 8 weeks after surgery. The calvaria were removed for macroscopic and radiographic photodocumentation and processed for histomorphometric analysis of bone healing at the surgical site. Macroscopic, radiological, and microscopic analyses demonstrated the biocompatibility of the implanted collagen membranes, as indicated by the absence of infiltration and signs of inflammation at the surgical site. Histologically, discrete immature bone neoformation projecting from the margins of the defect was observed at the surgical site in ovariectomised groups when compared to the non-ovariectomised groups. The volume of newly formed bone was significantly higher in the non-ovariectomised groups (G1: 7.83% ± 1.32; G2: 21.33% ± 1.96; and G3: 22.83% ± 0.98) compared to the respective ovariectomised subgroups (G4: 3.16% ± 0.75; G5: 16.83% ± 0.98; and G6: 16.16% ± 0.75), thus demonstrating the deleterious effects of ovariectomy on bone homeostasis. Higher volumes of newly formed bone were observed in the groups receiving the membrane grafts (G2, G3, G5, and G6) compared to the control groups (G1 and G4). In conclusion, the bilateral ovariectomy compromises the ability to repair bone lesions grafted with osteoconductive biomaterials as in the case of collagen membranes derived from both bovine pericardium and intestinal serosa.

Introduction

Studies in the area of material and tissue engineering have explored the development of biomaterials that possess the osteogenic capacity necessary for their osseointegration at the recipient site, favouring bone healing of the fractures or deformities [1,2]. The adequate biomaterial for this purpose should stimulate a specific biological response that depends on some intrinsic

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properties of the material, such as electron distribution, three-dimensional arrangement, molecular conformation, piezoelectric properties, porosity, and specific physicochemical properties.

As they meet these requirements, polymeric biomaterials have become important tools in reconstructive therapies of bone injuries [3]. In addition to their biocompatibility, natural polymers such as collagen, proteoglycans, glycosaminoglycans, and elastin participate in the control of tissue structure and in cell phenotype regulation, simulating the extracellular matrix [4,5]. Collagen as a biomaterial has several advantages for clinical application because of its biocompatibility, biodegradation, and weak antigenicity; in addition, collagen can be easily manipulated into different shapes [5–7].

Bovine and equine collagens, especially type I collagen, are frequently used due to their abundance and because they permit cell proliferation [7]. Collagen membranes derived from bovine pericardium can be used in bone repair due to advantages such as pliable texture, biodegradability, and properties of inducing deposits of calcium phosphate in a stage similar to bone mineralisation. Membranes derived from small intestinal submucosa have low immunogenicity and stimulate angiogenesis through vascular endothelial growth factor, allowing cell growth by nutrient diffusion. Furthermore, these membranes can undergo changes in their chemical properties by alkaline hydrolysis, which results in positively or negatively charged matrices. These modifications can improve the physiological properties of the material compared to native collagen by introducing dielectric properties, and they are also important for cell adhesion on culture substrates and mineralisation [6,8–11]. The possibilities of structural modifications of these membranes are an advantage compared to some marketed biomaterials. However, the bone health status is essential to permit the osseointegration of biomaterials and to obtain satisfactory results [6,12]. In this respect, some factors such as low calcium ingestion, smoking, alcoholism, physical inactivity, and hormone-related disorders due to gonadal hormone deficiency can compromise the success of implants [6,13–15]. Therefore, the objective of the present study was to evaluate the osteogenic feasibility of collagen membranes derived from bovine pericardium and intestinal serosa for bone neoformation during the repair of cranial defects in ovariectomised rats.

Materials and methods

Preparation of the polymeric matrices

Bovine intestinal serosa and bovine pericardium were exhaustively washed with 0.9% saline and distilled water. The serosa and pericardium, subsequently called matrix, were then submitted to alkaline treatment as follows: the matrix was immersed in an alkaline solution (hydrolysis) for 24 h at a temperature not exceeding 25 °C. Next, the matrix was equilibrated in another solution containing Na^+, K^+, and Ca^{2+} sulfates and chlorides. Excess salts were removed by washing in 3% boric acid and deionised water, followed by 0.3% ethylenediaminetetraacetic acid (EDTA) and deionised water [16]. After hydrolysis, the matrix was equilibrated in 0.01 mol L^{-1} H_3PO_4 for 24 h for swelling, and it was then lyophilised and stored at 25 °C. Mineralisation of the in vitro matrices was performed by alternating immersion in 0.2 mol L^{-1} $CaCl_2$, pH 7.4, and 0.12 mol L^{-1} Na_2HPO_4, pH 9.0, for 30 min each at 37 °C. This procedure was repeated six times. After mineralisation, the matrices were washed in deionised water and lyophilised [17], thus obtaining the definitive structural configuration of these matrices (Fig. 1A and B). After lyophilisation, the mineralised matrices were cut into samples measuring 5 mm in diameter for implantation into cranial bone defects of rats.

Membranes characterisation

The evaluation of the in vitro mineralisation was performed by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDX).

Differential scanning calorimetry. The data were obtained under a nitrogen atmosphere (80 mL min^{-1}) using a DSC-2010 (TA Instruments. New Castle, DE, USA) with a heating rate of 10 °C min^{-1}, between 5 and 120 °C, and a sample size of about 15 mg.

Thermogravimetric analysis. This was carried out using a TGA Q-50 (TA Instruments, New Castle, DE, USA). Heating was performed in a platinum pan in synthetic airflow (90 mL min^{-1}) at a rate of 10 °C min^{-1} up to 800 °C. The sample weight was in the range of 9–10 mg.

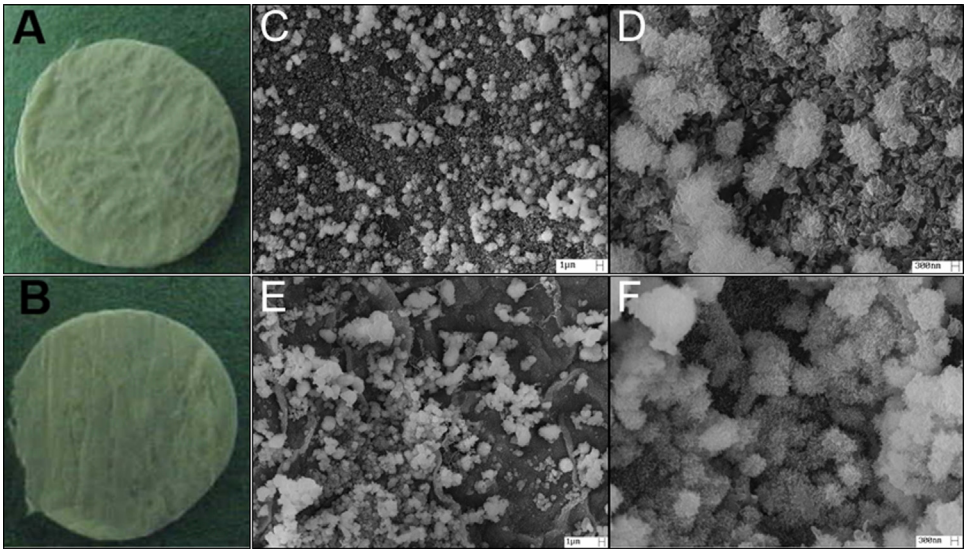

Fig. 1. Mineralised bovine pericardium-derived membrane: (A) digital photography and (C and D) SEM of the surface. Mineralised bovine intestinal serosa-derived membrane: (B) digital photography and (E and F) SEM of the surface. (C and E) 5000× and (D and F) 20,000×.