that PDCM cross-linked with different concentrations of GA (0.01%, 0.05%, and 3%) could retard the resorption rate in tissue and still preserve its biocompatibility. An in vitro study of PDCM co-cultured with human gingival fibroblasts (HGF) and human periodontal ligament fibroblasts (HPLF) presented the non-cytotoxic character of PDCM.[3] Another study indicated that the degradation rate of PDCM in osseous defects of rats is affected inversely by the cross-linking concentration of GA. PDCM cross-linked with 3% GA possessed the best quality for making spaces and inducing osteogenesis .[4]

In a study of cellular immunity elicited by PDCM, we selected anti-rat CD4 and CD8 monoclonal antibodies to detect the ratio of CD4+/CD8+ cells.[5] We learned that PDCM might elicit a delayed type of hypersensitivity in the early stage of wound healing. The ratio of CD4+/CD8+ cells was 2/1. Since PDCM is an xenogenic grafting protein, its MHC class II character may lead to either tissue repair or destruction. The purpose of this subsequent study was to use an immunohistochemical method to localize postulated adhesion molecules (α_2, α_3, and $\alpha_6\beta_1$), and 1 adhesive molecule, CD11b, on granulocytes at the interface of PDCMs and adjacent connective tissue. We hoped to investigate the tissue integration characteristics of PDCM and explain the possible trend of the effect of PDCM on tissue repair and proper GTR results.

MATERIALS AND METHODS

Twenty Wistar rats were used in this study. The experimental animals (wt 300 g) were anesthetized with phenobarbital sodium at 0.25 ml plus 1 ml normal saline by intraperitoneal injection. PDCM was submucosally implanted on the upper left vestibular area. A horizontal incision was made on the attached gingiva. Two vertical incisions were added in order to open a rectangular flap. Three percent GA-cross-linked PDCM was trimmed and embedded under the flap. Primary closure was achieved. A sham operation was performed on the upper right side without implantation of PDCM to serve as the control.

According to our timetable, 2 rats were euthanized with ether at 1, 2, 3, 5, 7, 10, 14, 21, 28, and 42 days after surgery. Specimens were harvested and embedded using OCT compound, freeze-dried, and preserved below –80°C.

Immunohistochemical staining

Freeze-dried specimens were sliced into 4-μm-thick sections. The avidin-biotin peroxidase complex method (ABC method) was performed by using anti-integrin α_2 antibody, anti-integrin α_3 antibody, anti-integrin $\alpha_6\beta_1$ antibody, and anti-CD11b antibody to localize adhesion molecule α_2, α_3, $\alpha_6\beta_1$, and CD11b expressions on granulocytes. The stained specimens were fixed with periodate-lysine-paraformaldehyde (PLP) at room temperature for 10 min. After washing with 0.05 M pH 7.4 PBS for 5 min 3 times, we used a solution of 30% H_2O_2: methanol: PBS in a 1: 3: 6 ratio to stop endoperoxidase activity at 4 °C for 30 min and than washed it with 0.05 M pH 7.4 PBS 3 times. One percent skim milk/PBS was applied at 37 °C for 30 min in order to fill voids in the tissue. Primary antibodies were then used at 4 °C overnight. The next day, secondary antibodies were applied at 37 °C for 1 hr after rinsing with 0.05 M ph7.4 PBS 3 times. Peroxidase/conjugated streptavidin was used at 37°C for 30 min, and it was washed with PBS 2 times. DAB at 0.05 gm was poured into a 10-ml PBS tube and dropped on the samples. We also used 30% H_2O_2 as the catalyst for 3-5 min. After washing with PBS for 5 min, hematoxylin was used as the counter stain for 30 s, and the sample was washed with tap water again for another 10 min. Specimens were then dehydrated with ethylene alcohol and processed with xylene for light microscopic observation. The presentation of α_2 and α_3 is shown by the descriptive mean. However, the number of CD11b+ cells was measured by using a grid screen under a light microscope. Ten arbitrarily selected fields in an 1x 1-cm^2 area were enlarged 400X, and the positive cells were counted.

RESULTS

On the second day after surgery, the tissue showed positive reactions for integrin α_2 and integrin α_3. They appeared mainly in intercellular spaces and on the sur-