Evaluation of initial attachment of human gingival fibroblast cells to biodegradable membranes *in vitro* by light and scanning electron microscopy

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Abstract: Guided tissue regeneration procedures using resorbable membranes have become accepted therapy for treating periodontal defects. Resorbable collagen and synthetic polylactide and polyglycolide copolymer membranes have been found to support regeneration and preclude the need for surgical removal. This study was undertaken to assess and compare the initial attachment of human gingival fibroblast cells to four collagen-based membranes (fascia lata, fascia temporalis, dura mater, and Type I bovine collagen) and a synthetic polylactic acid-based membrane (resolut®). Human gingival fibroblasts were grown from explants of normal tissue obtained during surgical reduction of retromolar tissues. Membrane specimens were placed in separate culture wells and incubated with fibroblasts for one hour. The number of adherent cells was evaluated by light microscopy using an ocular grid system and detailed examination was performed by scanning electron microscopy. The results of evaluation by light microscopy indicated that initial cell attachment was significantly less in the polylactic acid-based membrane group than in the collagen-based membrane groups ($P < 0.01$). However, no significant differences were found among the collagen membrane groups in terms of fibroblast attachment ($P > 0.01$). Scanning electron microscopy examination of fibroblasts cultured directly on barrier membranes indicated that the collagen-based membranes appeared to facilitate cell attachment, whereas the polylactic acid-based membrane exhibited a morphology that was not conducive to attachment of human gingival fibroblasts. Based on these limited *in vitro* results, it appears that collagen-based membranes offer greater potential than polylactic acid-based membranes for guided tissue regeneration at surgical sites. (J. Oral Sci. 41, 57-60, 1999)

Key words: Fibroblast; initial attachment; biodegradable membranes; guided tissue regeneration.

Introduction

The primary goal of the guided tissue regeneration (GTR) technique is to provide predictable regeneration of the periodontium by allowing repopulation of periodontal ligament cells onto exposed root surfaces, preventing ingrowth of gingival connective tissue and epithelial cells (1-4). A variety of resorbable and non-resorbable barrier membranes have been utilized as regenerative materials based on the principles of GTR. To avoid a second surgical operation solely for removal of non-resorbable membranes, several resorbable membranes have been developed, of which collagen and polylactic acid membranes have shown promising results (5-10).

Collagen is the most important structural protein component of the human body and an essential constituent of the periodontium for maintaining organic union between the bone and the teeth (11). *In vitro* experiments have determined that human gingival and periodontal ligament fibroblasts show continued growth on collagen materials (11,12). Recently, therefore most emphasis has been placed on the use of collagen-based membranes.

In cases where primary wound closure over a barrier membrane is not possible, cellular attachment to and migration over the barrier surface are critical to achieving wound closure and flap stabilization (13). The aim of the present study was to examine the ability of human gingival fibroblast (HGF) cells *in vitro* to produce initial attachment to five GTR membrane materials. Obviously, it is highly desirable to find a membrane barrier that can facilitate both osseous regeneration and migration of connective tissue cells to effect complete closure of overlying soft tissue defects.

Materials and Methods

Solvent-dried collagen-based membranes fascia lata, fascia temporalis, and dura mater were of human origin (Tutoplast, Biodynamics, Erlangen, Germany), and highly cross-linked Type I absorbable collagen membranes were of bovine origin (Colla-Tec, Inc., Plainsboro, NJ, USA). The polylactic/polyglycolide acid (PLA/PGA) copolymer was resolut® (Gore-Tex® Regenerative Materials, Flagstaff, AZ, USA). All were obtained commercially

Cell Culture

Fibroblasts were obtained from explant cultures of healthy human gingival tissues that were excised from retromolar gingiva during retromolar surgery. Gingival tissues were minced into small pieces (less than 0.5 mm), then plated and cultured according to the procedure described by Brunette et al. (14). Dulbecco's Modified Eagle Medium (DMEM) (Sigma, St. Louis, MO, USA) containing 15 % fetal calf serum (FCS) and 100 µg/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamycin and 2.5 mg/ml amphotericin B (Fungizone) was used for cell culture. Cells were cultured in a humidified atmosphere of 5 % CO₂ in air at 37°C until confluent monolayers were obtained. In the culture wells, old medium was replaced with fresh medium every 3 to 4 days for 1 week. Fibroblasts were separated for subculture with 0.25 % trypsin. Upon reaching confluence after about two weeks, coverslips were removed and the cultures dispersed with 0.25 % trypsin and Dulbecco salt solution, pH 7.3, into plastic tissue culture flasks. After the sixth passage cells were frozen at -70°C.

Cell Attachment

The 24 membrane specimens were placed in separate culture wells (Linbro plate) and incubated with 2.8×10^5 cells in 1 ml of DMEM in a humidified atmosphere of 95 % air and 5 % CO₂ for 1 h at 37°C. Unattached cells were resuspended every 15 min during the incubation period. After 1 h the membrane specimens were transferred to fresh wells, washed twice with phosphate buffered saline (PBS) and stained with Giemsa. Four samples of each membrane type were analysed by light microscopy (Olympus Vanox) for adhesion of human gingival fibroblasts. The number of attached cells per unit area was determined by an ocular grid (10×10 mm) system at a magnification of × 100. Quantitative results for each membrane specimens were obtained from four randomly chosen areas.

Two specimens from each membrane group were processed for evaluation by scanning electron microscopy (SEM). The cultures were rinsed in sucrose containing cacodylate buffer, fixed in 0.1 M cacodylate buffer containing 2 % glutaraldehyde, post-fixed in 1 % osmium tetroxide for 1 h, dehydrated in an ethanol series, critical point dried and coated with gold (Leitz AMR). They were then observed using low-temperature SEM (JEOL JSM 6400).

Statistical significance of differences in cell attachment was determined by analysis of variance and the Duncan multiple range test.

Results

Light Microscopy Observations

Statistical comparisons of the mean number of HGF cells attached to the membranes during 1 h are shown in Table 1. These measurements indicated significantly less initial cell attachment in the Resolut® group than in the other membrane groups ($P < 0.01$). However, no significant differences were found among the collagen-based membrane groups ($P > 0.01$). These cells were found to be not only attached, but in the process of spreading.

Table 1 Comparison of the mean number of cells on biodegradable membranes

Membrane groups	Mean ± SD
Fascia lata (n=4)	21.75 ± 0.26
Type I collagen barrier (n=4)	21.35 ± 0.49
Fascia temporalis (n=4)	21.25 ± 0.64
Dura mater (n=4)	22.55 ± 0.46
Resolut® (n=4)	12.80 ± 0.47*

* $P < 0.01$.

SEM Observations

Examination of the cultures by SEM showed the morphological characteristics of HGF cells and their attachment to the collagen and PLA membranes at 1 h. The HGF cells which adhered to the membranes showed variations in shape and size, however, no apparent differences were observed in the morphology of cells. The majority of cells cultured on the membranes showed a rounded morphology (Fig. 1:A1, A2, B1, B2, C1, C2, D1, D2, E1 and E2).

In the collagen-based membrane groups, the collagen fibers created mesh-like networks that overlapped each other (Fig. 1:A1, B1, C1 and D1). However, the surface texture of the membrane created in the PLA group consisted of solid fibers.

In some of the attached cells, the cytoplasmic processes were prominent, and appeared to mediate the adhesion of the cells to the membrane specimens (Fig. 1:A1 and A2).

Discussion

The results of this *in vitro* experiment showed that collagen- and PLA/PGA-based membranes were biocompatible for the initial attachment of HGF, but that the mean number of cells was greater over the surface of collagen-membrane barriers compared with those over the PLA/PGA membrane group.

Barrier membranes were used routinely in early GTR procedures and appear to have been ideally suited in cases where primary closure could be obtained over the wound site. However, primary wound closure is not always possible. Furthermore, even under ideal circumstances, injury to the gingiva is common during the healing phase. When recession does occur over a barrier, infection by pathogenic bacteria is likely to occur, and may result in a decreased regenerative response. If primary wound closure is not possible over membrane material, cellular attachment, especially HGF cell attachment, to the membrane surface is important in achieving wound closure (15).

Cellular attachment to the membrane-like substrate surface depends upon such intrinsic and extrinsic factors as the intracellular components of HGF and the physico-chemical nature of the membrane substrate (16). Thus, the biomaterial characteristics and design of membranes may play an important role in the attachment of fibroblasts. Resolut® membrane consists of an occlusive film with a bonded, randomly-oriented fiber matrix on each surface (4). This random organization

Fig. 1:A1 Fascia lata, original magnification $\times 400$

Fig. 1:A2 Fascia lata, original magnification $\times 3000$

Fig. 1:B1 Type I collagen barrier, original magnification $\times 400$

Fig. 1:B2 Type I collagen barrier, original magnification $\times 3000$

Fig. 1:C1 Fascia temporalis, original magnification $\times 400$

Fig. 1:C2 Fascia temporalis, original magnification $\times 3000$

Fig. 1:D1 Dura mater, original magnification $\times 400$

Fig. 1:D2 Dura mater, original magnification $\times 3000$

Fig. 1:E1 Resolut®, original magnification $\times 400$

Fig. 1:E2 Resolut®, original magnification $\times 3000$

of the membrane structure may decrease the attachment potential of fibroblasts. The structure of collagen-based membranes may allow more rapid attachment of fibroblast cells.

In a previous study, Payne et al. (15) used SEM to evaluate the ability of HGF cells *in vitro* to migrate along chemotactic gradients over three different GTR barrier materials: expanded polytetrafluoroethylene (ePTFE), polylactic acid and calcium sulfate. They showed that the mean migration distance over calcium sulfate was significantly greater than that over the polylactic acid membrane. The present study indicated that the calcium sulfate substrate appeared to facilitate cell attachment and spreading, and polylactic acid membranes exhibited a morphology that was not conducive to migration.

Finally, *in vivo* wound healing involves the interaction of a variety of cell types, including mesenchymal, hematological and inflammatory cells; however, only gingival fibroblast cells were examined in the present study. In addition, most *in vitro* models do not consider the influence of growth factors derived from blood and blood clots.

Further research is now necessary to evaluate the organization of fibroblast cells on these resorbable membranes.

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