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Biocompatibility of various collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells

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Abstract: The aim of the present study was to evaluate the biocompatibility of differently cross-linked collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells. Four collagen membranes [BioGide® (BG), BioMend® (BM), Ossix® (OS) and TutoDent® (TD)] were tested. Cells plated on culture dishes (CD) served as positive controls. Six specimens of each membrane were incubated with (1) human PDL fibroblasts [2×10^4 cells] ($n = 24$), and (2) human osteoblast-like cells (SaOs-2) [2×10^4 cells] ($n = 24$) under standardized conditions. After 7 days, adherent cells were stained with hematoxylin and counted using a reflected light microscope and the cell density per square millimeter was calculated. Additionally, cell morphology was investigated using scanning electron microscopy (SEM). Cell counts were presented as means and standard deviations (cells/mm^2) and analyzed for statistical difference using the Wilcoxon test: (1) CD (434 ± 76) > BG (64 ± 19) = OS (61 ± 8) > TD (44 ± 4) > BM (12 ± 5); (2) CD (453 ± 92) > BG (94 ± 46) = TD (84 ± 49) > OS (41 ± 23) > BM (0). SEM examination revealed that PDL fibroblasts adherent on BG, TD and OS appeared spindle-shaped and flat, like cells on CD. SaOs-2 osteoblasts adherent on CD were star shaped and flat, but mostly round in shape on BG, OS and TD. BM appeared to be incompatible with the attachment and proliferation of SaOs-2 cells; however, a few PDL fibroblasts were found in a round shape. Within the limits of the present study, it was concluded that (i) BG, TD and OS promoted, and (ii) BM inhibited the attachment and proliferation of human PDL fibroblasts and human SaOs-2 osteoblasts.

Guided tissue regeneration (GTR) has nowadays become an essential therapeutic procedure not only for the treatment of periodontal bone defects but also for bone- and peri-implant defects, and for bone augmentation procedures prior to implant placement. In the latter situation, it is sometimes termed guided bone regeneration (GBR) or guided bone augmentation (GBA) (Hämmerle & Karring 1998; Hämmerle & Lang 2001). The technique is based on the concept of preventing the apical downgrowth of the gingival epithelium inside the osseous defect, creating a secluded space that can be colonized by regenerative potential cells, such as PDL fibroblasts, cementoblasts and bone cells (Dahlin et al. 1988; Karring et al. 1993). A material that is used as a barrier for GBR/GTR has to satisfy some physicochemical characteristics to provide for biocompatibility, tissue integration, cell-occlusivity, space making ability, and also ease of use in the clinic (Gottlow 1993). The first generation of membranes was non-resorbable, mostly made from expanded polytetrafluorethylene (ePTFE). The ability of the ePTFE membranes to serve as devices for periodontal regeneration has been demonstrated in animal studies (Gottlow et al.

1984; Caffesse et al. 1990). Human histological data and the results from controlled clinical studies corroborated the results from these experimental studies (Nyman et al. 1982; Gottlow et al. 1986; Cortellini & Bowers 1995). However, one limitation of non-resorbable membranes is the need for a second surgery to remove the barrier. This may injure the obtained regenerated tissue, since it is evident that flap elevation results in a certain amount of crestal resorption of the alveolar bone (Pihlstrom et al. 1983). Furthermore, early spontaneous exposure to the oral environment and subsequent bacterial colonization have been reported to be common problems of non-resorbable membranes, which could necessitate their premature retrieval (Selvig et al. 1992; Tempro & Nalbandian 1993). In order to overcome these problems, a variety of synthetic resorbable materials, such as polylactid and polyglycolic acids, have been used as membrane barriers (Lorenzoni et al. 1998; Kohal et al. 1999). Recently, many investigations reported on the use of products derived from types I and III porcine or bovine collagen. The results from controlled clinical trials provide clear evidence that the application of both non-resorbable and resorbable membranes results in comparable clinical outcomes (Cortellini et al. 1996; Caffesse et al. 1997). Collagen membranes are resorbed by the enzymatic activity (collagenase) of infiltrating macrophages and polymorphonuclear leukocytes (Tatakis et al. 1999). To prolong their resorption, various cross-linking techniques, such as ultraviolet light, glutaraldehyde, diphenylphosphorylazide or hexmethylenediisocyanate have been used (Kodama et al. 1989; Minabe et al. 1989; Quteish & Dolby 1992; Brunel et al. 1996; Zahedi et al. 1998; Bunyaratavej & Wang 2001). The results from animal studies have demonstrated that the degradation of cross-linked collagen membranes was significantly slower compared with non-cross-linked membranes (Pitaru et al. 1988; Paul et al. 1992). Furthermore, it has been shown that the resorption rate depends upon the degree of cross-linking (i.e., the higher the degree of cross-linking, the longer the resorption rate) (Brunel et al. 1996). However, little is known about the influence of these cross-linking techniques on the attachment and proliferation of regenerative potential cells. The aim of the present study was therefore to evaluate the biocompatibility of differently cross-linked collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells.

Material and methods

Membranes examined

Four commercially available GBR/GTR collagen membranes were tested: (1) Bio-Gide® (BG) (Geistlich Biomaterials, Wolhusen, Switzerland) (non-cross-linked porcine types I and III collagen); (2) BioMend® (BM) (Sulzer Medica, Colla-Tec, Inc., Plainsboro, NJ, USA) (glutaraldehyde cross-linked bovine type I collagen); (3) Ossix® (OS) (3i, Colbar R&D Ltd, Ramat Husharon, Israel) (enzymatic-cross-linked bovine type I collagen); and (4) TutoDent® (TD) (Tutogen, Carlsbad, CA, USA) (non-cross-linked bovine type I collagen).

Cell isolation

Periodontal ligament fibroblasts were derived from a 22-year-old woman undergoing lower third molar extractions based on signed consent. The culture procedure was conducted according to Mailhot et al. (1995). Briefly, teeth were extracted under strict aseptic conditions. The mid-third portion of the root surfaces was collected carefully by scraping with a surgical scalpel in the sterile atmosphere of a laminar flow chamber and placed on a petri dish with 2 ml of Dulbecco's modified eagle medium (DMEM) (Cambrex Bio Science, Verviers, Belgium) supplemented with 1% penicillin/streptomycin and 15% fetal bovine serum (Gibco, BRL, Life Technologies GmbH, Karlsruhe, Germany). Culturing was set at 37°C in a humified atmosphere of 95% air and 5% CO_2. The medium has been changed every 2–3 days. When emigrating fibroblast-like cells (PDL) became confluent around most of the tissue fragments, the medium was removed and the cell layer washed with phosphate-buffered saline, then ethylenediaminetetraacetic was added, and incubation continued for 20 min. The cells detached by this procedure were stored in liquid nitrogen for use in our experiment.

Cell cultures

Twelve specimens of each membrane ($n = 48$) with a diameter of 10 mm were washed in PBS for 15 min, fixed in minusheet-rings (No. 1300, Minucells and Minutissue Vertriebs GmbH, Bad Abbach, Germany) and placed into 24-well plates (Lap Tek Chamber Slide, Nalge Nunc, Naperville, IL, USA). Six specimens of each group were covered with either a solution of (1) human PDL fibroblasts (fourth passage, 2×10^4 cells suspended in 2 ml of DMEM and 10% fetal bovine serum supplemented with 1% penicillin/streptomycin) ($n = 24$), or (2) human SaOs-2 osteoblasts [sixth passage, 2×10^4 cells suspended in 2 ml Mc Coy's 5A medium (Gibco No. 21017-025, Life Technologies GmbH, Karlsruhe, Germany) supplemented with 1% penicillin/streptomycin] ($n = 24$). In case of the bilayered BG and TD membranes, SaOs-2 osteoblasts were cultivated on the porous surface. Cells plated on culture dishes (CD) served as positive controls. The incubation period was 7 days. Culturing was set at 37°C in a humified atmosphere of 95% air and 5% CO_2. The medium was changed every 2–3 days. Additionally, four samples of each membrane ($n = 32$) were cultivated under the same conditions for scanning electron microscopy (SEM). After incubation, the specimens were gently washed with phosphate-buffered saline to remove cells not attached to the surface.

Microscopic analysis

After rinsing with PBS, the specimens were fixed with paraformaldehyde (3%) for 60 min and stained with hematoxiline. Cells were counted using a reflected light microscope (Leitz Orthoplan, Leitz, Wetzlar, Germany) (magnification × 200) and a counting grid (exactly 0.25 mm^2). Six areas on each surface were scanned and all stained cells counted out. All the samples were investigated by one blinded examiner, and the mean cell density per square millimeter was calculated.

SEM observation

After rinsing with PBS and fixing for 30 min with 4% glutaraldehyde in 0.15 M PBS phosphate buffer (pH = 7.4) at room temperature, the membranes were washed in 0.15 M PBS for 15 min. The specimens were dehydrated in increasing concentrations of alcohol (from 40 to 100%, 10% steps). Then the specimens were dried in hexamethyldisilazane, sputter coated with

gold and examined using SEM (Scanning Microscope DSM 950, Zeiss, Germany). All the samples were investigated by one blinded examiner.

Statistical analysis

A software package (SPSS 11.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The number of cells on an area of 0.25 mm² was multiplied by 4 to indicate the cell density per square millimeter for each specimen. Mean values and standard deviations (SDs) were then calculated for each group. Comparisons between the groups were performed by the Wilcoxon test, and differences were considered to be significant when $P<0.05$.

Results

Assessment of cell numbers

During the experimental period, there were no signs of any bacterial or fungal contamination of the well chambers. Data were presented as mean ± SD. The highest number of PDL fibroblasts per square millimeter was seen on CD (434 ± 76). In comparison with the positive control, BG (64 ± 19), OS (61 ± 8) and TD (44 ± 4) showed statistically significant fewer cells ($P=0.028$, respectively). The difference between BG and OS was statistically non-significant ($P=0.917$). TD exhibited significantly fewer cells per square millimeter than BG and OS ($P=0.046$, $P=0.028$, respectively). There was, however, a statistically significant decrease in the number of PDL fibroblasts that attached and proliferated on BM (12 ± 5) when compared with CD, BG, OS and TD ($P=0.028$, respectively) (Fig. 1). In SaOs-2 culture, the highest cell density per square millimeter was also calculated for the positive control (CD) (453 ± 92). This was followed by BG (94 ± 46), TD (84 ± 49) and OS (41 ± 23) with statistically significant fewer cells per square millimeter ($P=0.028$, respectively). The difference between BG and TD was statistically non-significant ($P=0.6$). OS exhibited significantly fewer cells per square millimeter than BG and TD ($P=0.028$, respectively). However, there were no SaOs-2 cells detectable on BM (0) ($P=0.028$, respectively) (Fig. 2).

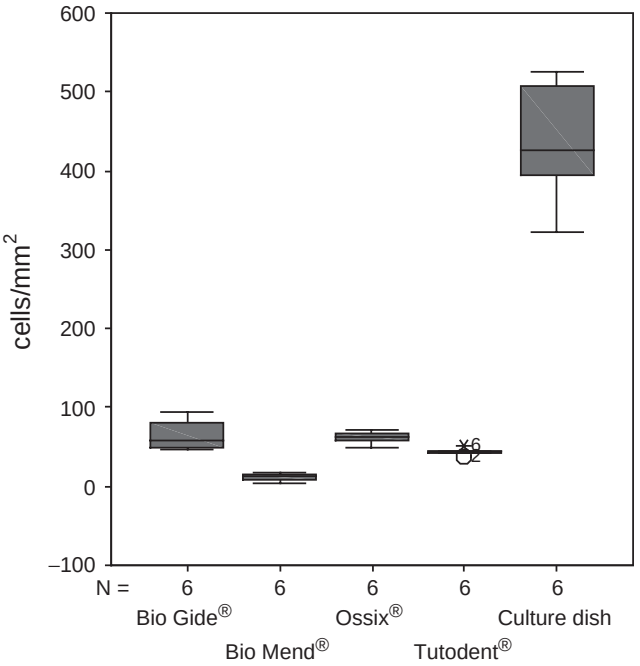

Fig. 1. Boxplots with outliners for the medians and Q1–Q3 quartiles of cell density (cells/mm²) on different collagen membranes, evaluated after 7 days incubation in a human PDL fibroblast suspension. Lines below and above box plots = min, max.

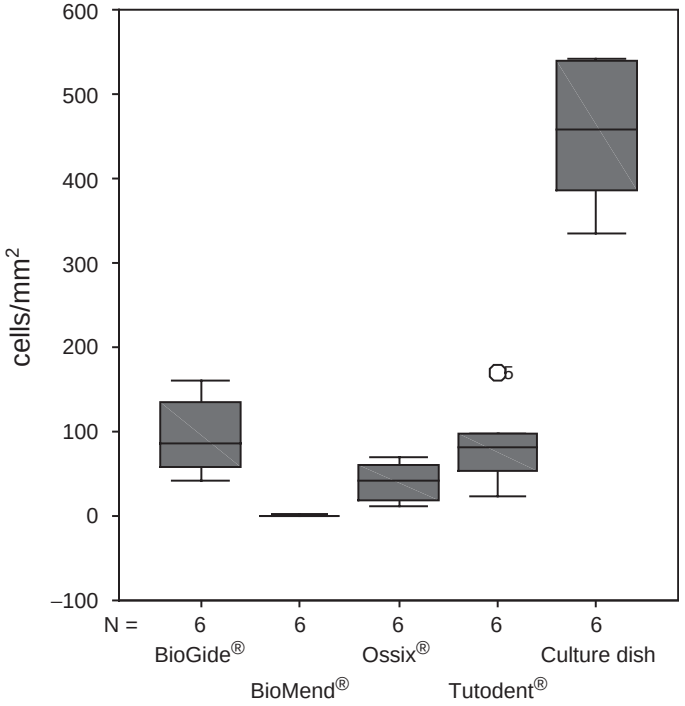

Fig. 2. Boxplots with outliners for the medians and Q1–Q3 quartiles of cell density (cells/mm²) on different collagen membranes, evaluated after 7 days incubation in a human osteoblast-like cell suspension. Lines below and above box plots = min, max.

Cell morphology

SEM examination after 7 days revealed different cell morphology of PDL fibroblasts and SaOs-2 osteoblasts grown on CD. PDL fibroblasts were elongated and spindle shaped, whereas SaOs-2 osteoblasts exhibited a star-shaped appearance. On CD, both cell types seemed to be flat with cytoplasmatic extensions and lamellopodia approaching confluency (Figs 3a and 4a).

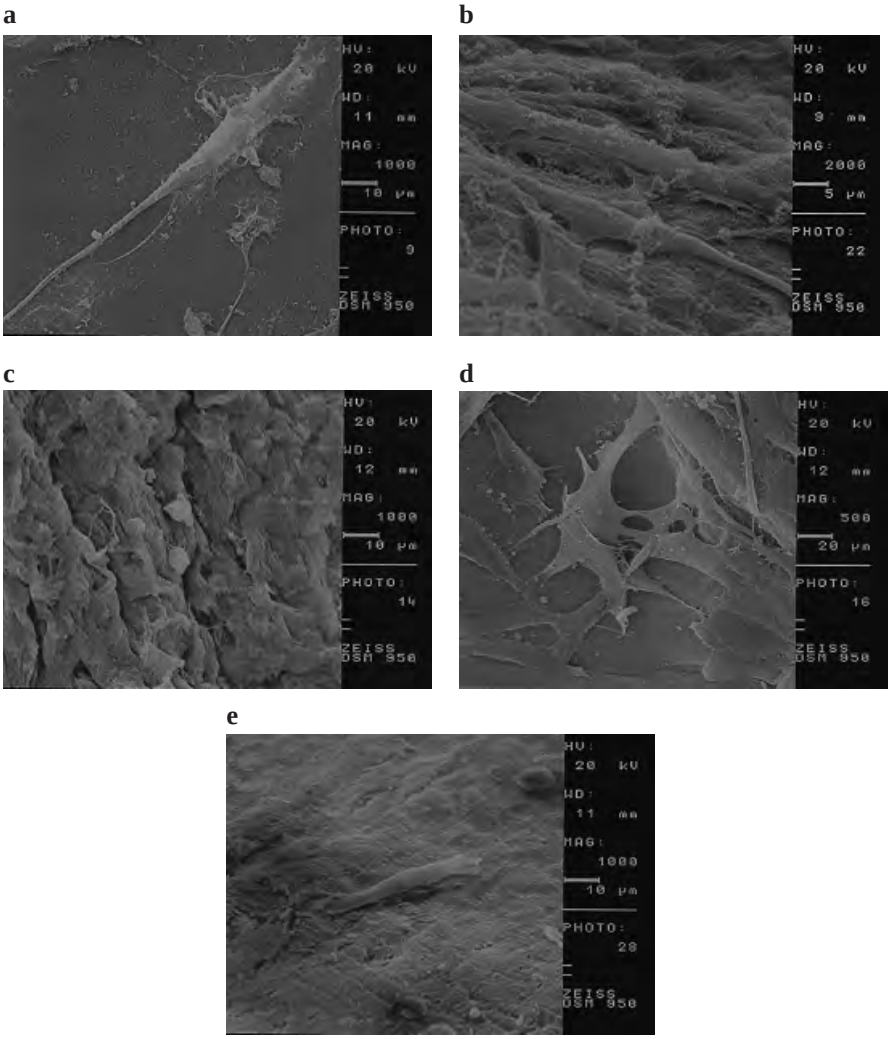

Fig. 3. Scanning electron microscope view of human PDL fibroblasts adherent to culture dish and various collagen membranes after 7 days. (a) Culture dish (1 : 1000), (b) BioGide® (1 : 2000), (c) BioMend® (1 : 1000), (d) Ossix® (1 : 500), and (e) Tutodent® (1 : 1000).

PDL fibroblasts adherent on BG, OS and TD were similar in shape (Fig. 3b, d, e). In contrast, BM appeared to be incompatible with PDL fibroblast attachment and proliferation as only a few round and no spindle-shaped PDL fibroblasts could be observed (Fig. 3c). SaOs-2 osteoblasts adherent on BG, OS and TD were mostly round in shape without cytoplasmatic extensions and lamellopodia (Fig. 4b, d, e). BM appeared to be incompatible with cellular attachment and proliferation since no SaOs-2 osteoblasts could be observed (Fig. 4c).

Discussion

The present study was designed to evaluate human PDL fibroblast and human SaOs-2 osteoblast-like cell attachment and morphology when exposed to types I and III collagen membranes. Collagen has been shown to be advantageous over other synthetic materials used for bioabsorbable membranes since it plays an active role in coagulum formation, is chemotactic for PDL fibroblasts and gingival fibroblasts and is a major component of the periodontal connective tissue (Postlethwaite et al. 1978; Yaffe et al. 1984; Hutmacher et al. 1996; Locci et al. 1997). Within the limits of this *in vitro* study, the mean number of attached and proliferated PDL fibroblasts was greatest on CD, followed by BG, OS and TD, with the least amount of attachment and proliferation noted on BM. Similar results were noted for the mean number of attached and proliferated SaOs-2 osteoblasts. The mean number of osteoblast-like cells was greatest on CD, followed by BG, TD and OS. In contrast, BM appeared to be incompatible with cellular attachment and proliferation since no cells could be observed. Thus, it may be assumed that all tested collagen membranes limit or even inhibit the attachment and proliferation of both human PDL fibroblasts and human SaOs-2 osteoblasts.

These findings are partially consistent with the results from previous studies evaluating the initial attachment of PDL fibroblasts and osteoblasts to various GBR membranes (Takata et al. 2001a, 2001b; Wang et al. 2002). Takata et al. (2001a) evaluated the attachment, proliferation and differentiation of rat-derived PDL fibroblasts. It was reported that the initial cell attachment (at 1.5 h) on BM was significantly lower than on a bovine type I atelocollagen membrane (TG), polylactid/polyglycolic acid membranes, an e-PTFE membrane or CD. However, BM showed linear growth of PDL cells throughout the study period, with a significantly higher number of cells at 5 days than at 1.5 h. Wang et al. (2002) evaluated osteoblast (MC3T3-E1 mouse osteoprogenitor cells) attachment at both earlier (1.5 h) and later (24 h after) time periods. A cellulose ester mixture (MF) exhibited a statistically significant higher cell attachment than all other tested barriers. In particular, the mean cell number per square millimeter for MF was 27.5 ± 2.1 at 1.5 h and 67.6 ± 3.6 at 24 h, and for BM cell number per square millimeter was 14.5 ± 1.4 at 1.5 h and 15.4 ± 0.9 at 24 h. In contrast to the results of the present study, it was concluded that BM still enhances the early osteoblast attachment, since a similar amount of cells was also noted for polylactic and polyglycolic membranes. However, it was noted that on BM, the cells did not proliferate with time. The cell number remained at the same level after initial attachment. This may be attributed to the influence of different components or structures noted in BM. As mentioned above, physical and chemical techniques to increase cross-linking have been used to control the rate of collagen biodegradation (Kodama et al. 1989; Minabe et al. 1989; Quteish & Dolby 1992; Brunel et al. 1996; Zahedi et al. 1998; Bunyaratavej & Wang 2001). Cross-linking with glutaraldehyde resulted in a decreased membrane

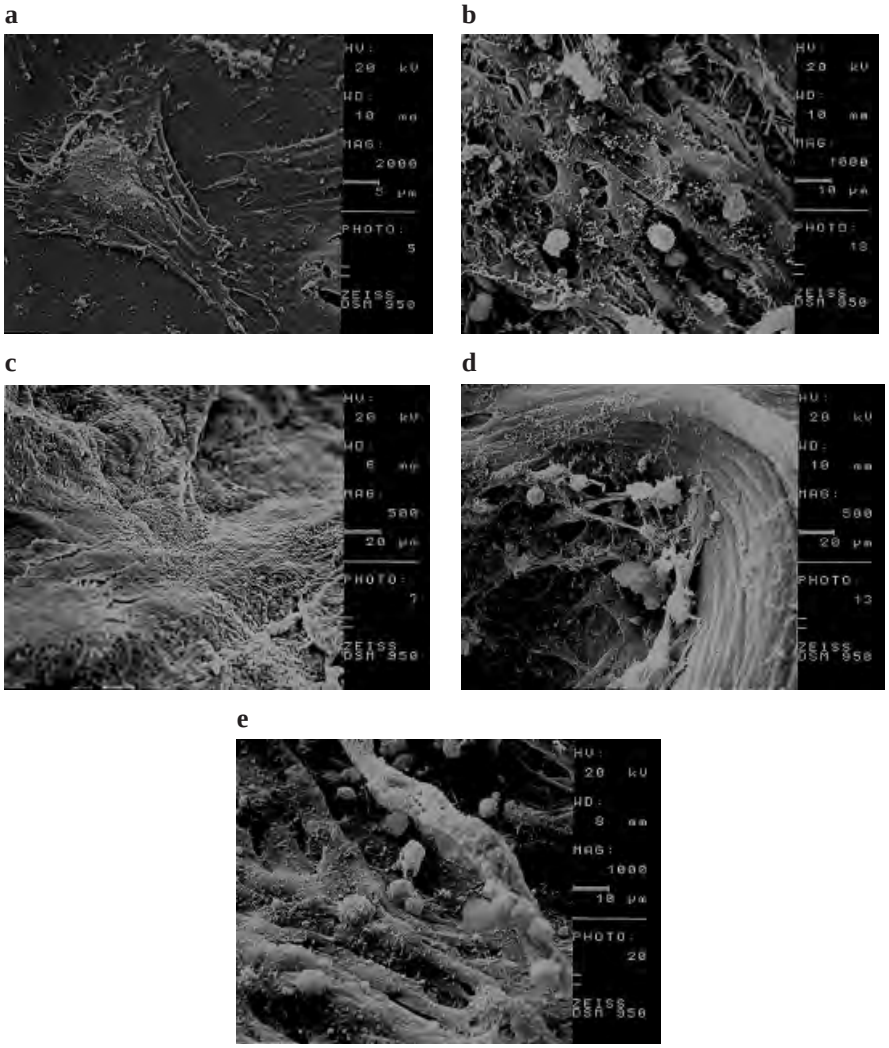

Fig. 4. Scanning electron microscope view of human SaOs-2 osteoblasts adherent to culture dish and various collagen membranes after 7 days. (a) Culture dish (1 : 2000), (b) BioGide® (1 : 1000), (c) BioMend® (1 : 500), (d) Ossix® (1 : 500), and (e) Tutodent® (1 : 1000).

biocompatibility due to cytotoxic effects (Speer et al. 1980; Wiebe et al. 1988). Takata et al. (2001b) evaluated the biological effects of GBR membranes on osteoblastic (MC3T3-E1) cell migration (Takata et al. 2001b). It was reported that BM, TG, and MF showed a migration rate equal to CD on which cells generally grow favorably. However, cell migration after 5 days was significantly lower on BG. The discrepancies noted in these results may be explained by different cell types being used. Takata et al. (2001a) used rat-derived PDL fibroblasts, while Takata et al. (2001b) and Wang et al. (2002) used a mouse osteoprogenitor cell line (MC3T3-E1). In the present study, we used human PDL fibroblasts and human osteosarcoma-derived SaOs-2 cells that have been well characterized as osteoblast-like cells (Murray et al. 1987; Rodan et al. 1987). However, transformed cell lines have their own limitations, as some of the cell characteristics are different from those of primary cells. Nevertheless, in long-term *in vitro* mineralization studies, normal human osteoblast cultures responded in a similar way to implant surfaces such as SaOs-2 cells, but with approximately two-third less calcification (Ahmad et al. 1999). In this context, it is important to point to the results of a previous study, which have shown that BG exhibited an excellent cytocompatibility in cultures of human PDL fibroblasts and human SaOs-2 osteoblasts (Alpar et al. 2000). These findings, coupled with the results of the present study, seem to indicate that the attachment and proliferation of the above-mentioned cells (Takata et al. 2001a, 2001b; Wang et al. 2002) differ from that of human-derived cells. The present results have also demonstrated that the mean numbers of both PDL fibroblasts and SaOs-2 cells on CD were significantly higher than on all tested barrier materials. These findings are consistent with previous studies that reported the greatest amount of cells attached on CD as compared with other bioabsorbable membranes (Takata et al. 2001a; Wang et al. 2002). Surface topography also plays an important role in the adhesion of cells (Brunette 1988). Rough or textured porous surfaces have been considered to promote cell attachment. It has been shown that fibroblasts prefer smooth or finely textured surfaces, while osteoblast-like cells attach more to rough surfaces, allowing increased mineralization (Bowers et al. 1992; Kononen et al. 1992). Moreover, cell morphology can be regarded as an indicator of the affinity of the cells to a substratum. Flat cells are firmly attached by means of numerous attachment extensions and lamellopodia (Trylovich et al. 1992). Furthermore, anchorage-dependent cells exhibiting a rounded appearance have been reported to divide at a lower rate than cells exhibiting a flattened morphology (Folkman & Moscona 1978; Archer et al. 1982). These findings, coupled with the results of the present study, which have shown that PDL fibroblasts adherent on BG, OS and TD were flattened, whereas adherent SaOs-2 osteoblasts were more round in shape, seem to indicate that these membranes offer surface conditions more favorable to the attachment and proliferation of human PDL fibroblasts than to SaOs-2 osteoblasts. Furthermore, BM seemed to be incompatible with cell attachment and proliferation since only a few round PDL fibroblasts and no SaOs-2 osteoblasts could be observed. As mentioned above, cross-linking with glutaraldehyde may be a possible explanation for the decreased biocompatibility of this collagen membrane (Speer et al. 1980; Wiebe et al. 1988). Because no previously published data on the cytocompatibility of OS and TD are available, the present results cannot readily be compared with those of other studies. However, it must be pointed out that the results obtained by using an *in vitro* experimental model cannot recreate the complex interactions of cells *in vivo*. Further studies using

controlled experimental *in vivo* models are needed in order to verify the present results. In this context, an issue that has not been precisely determined is the ideal time period that the membrane should retain the barrier function in order to maximize the healing results.

Within the limits of the present study, it was concluded that (i) BG, OS and TD promoted, and (ii) BM inhibited the attachment and proliferation of human PDL fibroblasts and human SaOs-2 osteoblasts.

Acknowledgements: This study was supported by a grant of Geistlich Biomaterials, Wolhusen, Switzerland.

Résumé

Le but de l'étude présente a été d'évaluer la biocompatibilité de membranes collagène croisées différement dans des cultures de fibroblastes du parodonte et de cellules ressemblant à des ostéoblastes humains. Quatre membranes en collagène [BioGide® (BG), BioMend® (BM), Ossix® (OS), TutoDent® (TD)] ont été testées. Des cellules placées dans des boîtes de culture (CD) ont servi de contrôle positif. Six spécimens de chaque membrane ont été incubés avec 1) des fibroblastes du parodonte [2×10^4 cellules] ($n = 24$) et 2) des cellules ressemblant à des ostéoblastes humains (SaOs-2) [2×10^4 cellules] ($n = 24$) sous des conditions standards. Après sept jours, des cellules adhérentes ont été colorées avec de l'hématoxyline et comptées microscopiquement et la densité cellulaire par mm² a été calculée. De plus, la morphologie cellulaire a été jugée dans un MEB. Des comptages cellulaires ont été présentés en moyenne et déviation standard (cellules/mm²) et analysés pour les différences statistiques en utilisant le test de Wilcoxon 1) CD (434 ± 76)>BG (64 ± 19)=OS (61 ± 8)>TD (44 ± 4)>BM (12 ± 5); (2) CD (453 ± 92)>BG (94 ± 46)=TD (84 ± 49)>OS (41 ± 23)>BM (0). L'examination MEB a révélé que les fibroblastes adhérant sur BG, TD et OS semblaient plats et en fuseau, comme les cellules sur CD. Les ostéoblastes SaOs-2 adhérant sur CD étaient plats et en forme d'étoile mais majoritairement ronds sur BG, OS et TD. BM apparaîssait incompatible avec l'attache et la prolifération des cellules SaOs-2 bien que quelques fibroblastes ronds aient été trouvés. Dans les limites de l'étude présente, BG, TD, OS favoriseraient tandis que BM inhiberait l'attache et la prolifération des fibroblastes du parodonte et des ostéoblastes SaOs2.

Zusammenfassung

Das Ziel dieser Studie war, in Kulturen von menschlichen PDL-Fibroblasten und osteoblastenähnlichen Zellen die Biokompatibilität von verschieden vernetzten Kollagenmembranen zu untersuchen. Man testete vier Kollagenmembranen, BioGide® (BG), BioMend® (BM), Ossix® (OS) und TutoDent® (TD). Auf Kulturscheiben (CD) ausgestrichene Zellen dienten als Positivkontrollen. Danach inkubierte man unter standartisierten Bedingungen von jeder Membran sechs Exemplare mit (1) menschlichen PDL-Fibroblasten {2 × 104 Zellen} ($n = 24$) und mit (2) menschlichen osteoblastenähnlichen Zellen (SaOs-2) {2 × 104 Zellen} ($n = 24$). 7 Tage später färbte man die Membranen mit Hematoxylineblau ein, zählte unter einem Reflektionslichtmikroskop die anhaftenden Zellen und berechnete die Zelldichte pro mm². Mit einem SEM beurteilte man zusätzlich die Zellmorphologie. Die Zellzahl wurde als Mittelwert mit Standartabweichung (Zellen/mm²) aufgeführt, und für die statistische Auswertung kam der Wilcoxon-Test zur Anwendung: (1) CD (434 ± 76)>BG (64 ± 19) =OS (61 ± 8)>TD (44 ± 4)>BM (12 ± 5); (2) CD (453 ± 92)>BG (94 ± 46)=TD (84 ± 49) >OS (41 ± 23)>BM (0). Die SEM-Untersuchungen zeigten, dass die auf den BG, TD und OS anhaftenden PDL-Fibroblasten spindelförmig und flach aussahen, genauso wie die Zellen auf den CD. Die SaOs-2-Osteoblasten auf den CD waren sternförmig und flach, auf den BG, OS und TD aber meistens rund. Bei der BM schien eine Adhäsion und Proliferation von SaOs-2-Zellen unmöglich zu sein, man fand aber einige runde PDL-Fibroblasten. Trotz den eingeschränkten Interpretationsmöglichkeiten dieser Studie, schloss man, dass (i) die BG, TD und OS sowohl Adhäsion wie auch Proliferation von menschlichen PDL-Fibroblasten und SaOs-2-Osteoblasten fördern, während (ii) die BM sie hemmen.

Resumen

La intención del presente estudio fue evaluar la biocompatibilidad de diferentes membranas de colágeno relación cruzada con cultivos de fibroblastos humanos PDL y células humanas tipo osteoblastos. Se probaron cuatro membranas de colágeno [BioGuide® (BG), BioMend® (BM), Ossix® (OS), TutoDent® (TD)]. Como control se utilizaron células colocadas en discos de cultivo (CD). Se incubaron seis especímenes de cada membrana con (1) fibroblastos humanos PDL [2×10^4 células] ($n = 24$), y con (2) células humanas tipo osteoblastos (SaOs-2) [2×10^4 células] ($n = 24$) bajo condiciones estándar. Tras 7 días, se tiñeron las células adherentes con hematoxilina y se contaron usando un microscopio de luz reflejada y se calculó la densidad de células por mm². Además, se investigó la morfología celular usando SEM. Los recuentos celulares se presentaron como medias y desviaciones estándar (células/mm²) y analizadas para análisis estadístico usando el test de Wilcoxon: (1) CD (434 ± 76)>BG (64 ± 19)=OS (61 ± 8)>TD (44 ± 4)>BM (12 ± 5); CD (453 ± 92)>BG (94 ± 46)=TD (84 ± 49)>OS (41 ± 23)>BM (0). El examen SEM reveló que los fibroblastos PDL adherentes a BG, TD y OS aparecieron fusiformes y con forma plana, como las células en CD. Los osteoblastos SaOs-2 adherentes en CD tenían forma de estrella y eran planos, pero eran principalmente de forma redonda en BG, OS < y TD. BM pareció ser incompatible con inserción y proliferación de células SaOs-2, sin embargo se encontraron algunos fibroblastos PDL en forma redonda. Dentro de los límites del presente estudio, se concluye que (i) BG, TD y OS promovieron y (ii) BM inhibió la inserción y proliferación de fibroblastos humanos PDL y osteoblastos humanos SaOs-2.

要旨

本研究は、ヒトの PDL 線維芽細胞とヒトの骨芽細胞様細胞の培養において、異なる架橋を形成したコラーゲン・メンブレンの生体適合性を評価した。4つのコラーゲン・メンブレン［BioGide®(BG)、BioMend®(BM)、Ossix®(OS)、TutoDent®(TD)］を調べた。培養皿（CD）上にのせた細胞を陽性対照として用いた。各メンブレンの6つの検体を標準条件下で、（1）ヒト PDL 線維芽細胞[2x10 の 4 乗]（n=24）と（2）ヒト骨芽細胞様細胞（SaOs2）[2x10 の 4 乗](n=24)で培養した。7日後に付着細胞をヘマトキシリンで染色し、反射光顕微鏡を用いて細胞数を数え、mm²あたりの細胞密度を計算した。さらに細胞の形態をSEMによって調べた。細胞数については平均と標準偏差（細胞／mm²）を提示し、Wilcoxon 検定を用いて統計的有意差を分析した：(1)CD(434 ± 76)>BG(64 ± 19)=OS (61 ± 8)>TD(44 ± 4)>BM(12 ± 5)；(2) CD (453 ± 92)>BG(94 ± 46)=TD(84 ± 49)>OS(41 ± 23)>BM(0)。SEM検査では、BG、TD及びOSに付着したPDL繊維芽細胞は、CD上の細胞と同様に、紡錘形で平坦であった。CDに付着したSaOs2骨芽細胞は星形で平坦であったが、BG、OS及びTD上のものは大半が丸い形をしていた。BMは SaOs2 細胞の付着と増殖に不適合であるように思われたが、少しのPDL線維芽細胞は丸い形をしていた。本研究の制約内において、（i）BG、TD及びOSはヒトPDL線維芽細胞とヒトSaOs2骨芽細胞の増殖と付着を促進し、（ii）BMはそれを阻害すると結論した。

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