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Migration of osteoblastic cells on various guided bone regeneration membranes

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Abstract: To evaluate the biological effects of guided bone regeneration (GBR) barrier materials on osteoblastic cell migration, migration of mouse osteoprogenitor cells (MC3T3-E1) was examined, *in vitro*, on various membranes. Eight commercially available GBR membranes – bovine type I collagen (BioMend®; BM), porcine type I collagen (BioGide®; BG), bovine type I atelocollagen (Tissue Guide®; TG), polylactic acid (Epi-Guide®; EG), co-polymer of polylactic acid and polyglycolic acid (Resolute®; RL, Resolut XT®; RL-XT), expanded polytetrafluoroethylene (e-PTFE; Gore Tex®; GT) and co-polymer of cellulose acetate and nitrocellulose (Millipore filter®; MP) – were tested. A 3×5 mm section of the membrane was fixed to the bottom of a culture dish with double-sided adhesive tape, and half of the membrane was closely covered by PARAFILM® (American National Can™) to leave an unexposed area for cell migration. The border between exposed and unexposed areas was marked as a baseline of cell migration. Membranes were then plated with 3 ml of cell suspension at an initial density of 1×10^5 cells/ml in α-MEM culture medium with 10% fetal bovine serum and ascorbic acid. After a 5-hour incubation, non-attached cells were completely washed out with phosphate buffered saline and the PARAFILM® cover was removed. After 3 days cultivation, specimens were fixed with 10% buffered formalin and stained briefly with hematoxylin. The area of cell migration on a membrane was analyzed using a LA 500 Image Analysis System and migration area per unit length of the baseline (mm^2/mm) was compared among membranes. Results demonstrated that cell migration was greater in the order: RL>RL-XT, BM, TG, MP>EG, BG. Membranes except for BG, EG and GT showed the migration rate equal to or higher than a plastic culture cover slip (Celldesk®) ($P<0.01$) on which cells generally grow favorably. Only a small number of the cells attached to GT, and the net cell migration for the membrane could not be determined. These results indicate that GBR barrier materials *per se* may influence the process of bone regeneration *in vivo* through the effects of their presence on cell migration.

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Guided bone regeneration (GBR) has slowly become an acceptable method in clinical dentistry to facilitate augmentation of alveolar ridge defects, to promote implant wound healing, and to repair/regenerate implant defects. A variety of synthetic and naturally derived GBR barriers have been developed and tested with promising results (Hammerle 1999). The membranes are often used to create a space between the bone compartment and the overlying gingival flap. Even though the barriers may differ in composition and structure, they are all supposed to prevent epithelial and connective tissue cells, which migrate more quickly than bone cells, from invading the area where angiogenesis and osteo-

genesis must take place (Buser et al. 1990; Buser et al. 1996; Wang 1998). In addition, some barriers also act as a substrate for tissue migration and/or generation on both sides which could facilitate both the process of osseous regeneration and migration of connective tissue cells to effectively complete closure of overlying soft tissue defects (Payne et al. 1996).

Ideally, barriers should facilitate cell attachment and promote migration of the progenitor cells. In order for osteoblastic progenitor cells to proceed with the wound healing cascade, proliferation, differentiation and tissue maturation, cells need to adhere to a substrata first (Grinnell 1978). In our previous study, the initial attachment of osteoblastic cells on various GBR barriers was examined (Wang et al. 1997). Data from this study indicated that the membrane *per se* might influence cell attachment. In addition, the biological effects of guided tissue regeneration (GTR) membranes on periodontal tissue regeneration were analyzed (Takata et al., 2001). It was concluded that GTR barriers influence cell proliferation and differentiation in the process of periodontal tissue regeneration. Salonen & Persson (1990) also showed that epithelial cell migration along the Biopore® teflon membrane was significantly greater than along the Gore Tex periodontal materials. They suggested that the low protein-binding capacity and the rough-textured surface of the Gore Tex inhibit epithelial cell migration.

However, little is known about how the different composition and structures of membranes may influence osteoblastic migration, proliferation and differentiation. Therefore, the purpose of this study was to determine whether cellular migration of osteoblastic cells is affected by different composition and structures of the membranes used in today's GBR procedures.

Material and methods

Membranes examined

Eight commercially available GBR membranes were tested: bovine type I collagen (BioMend®; BM, Sulzer Calcitek Inc., CA, USA), porcine type I collagen (BioGide®; BG, Osteohealth Co., NY, USA), bovine type I atelocollagen (Tissue Guide®; TG, Koken, Tokyo, Japan), polylactic acid (Epi-Guide®; EG, THM Biomedical Inc., MN, USA), copolymer of polylactic acid and polyglycolic acid (Resolute®, RL; Resolut XT®, RL-XT; W.L.Gore & Associates, AZ, USA), expanded polytetrafluoroethylene (e-PTFE; Gore Tex®; GT, W.L. Gore & Associates) and co-polymer of cellulose acetate and nitrocellulose (Millipore filter®; MP, Millipore Co., MA, USA).

Cells

MC3T3-E1 cells, a mouse osteoprogenitor cell line, were maintained in α-MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics, and used for the migration assay between passage 10 to 15.

Migration assay

A 3×5 mm section of the membrane was fixed to the bottom of a culture dish with sterile double-sided adhesive tape (Fig. 1a), and half of the membrane was closely covered by PARAFILM® (American National Can™; Chicago, IL, USA) to leave an unexposed area for cell migration (Fig. 1b). The border between exposed and unexposed areas was marked as a baseline of cell migration (Fig. 1b). Membranes were then plated with 3 ml of cell suspension at an initial density of 1×10^5 cells/ml in α-MEM culture medium with 10% FBS and ascorbic acid (Fig. 1b). After 5 hours incubation, non-attached cells were completely washed out with phosphate buffered saline (PBS) and the PARAFILM® cover was removed (Fig. 1c). After 3 days cultivation, specimens were fixed with 10% buffered formalin and stained briefly with hematoxylin. Three specimens for each membrane were photographed under a magnification ×25. Using the prints, the area of cell migration was traced on a translucent paper and analyzed with an LA 500 Image Analysis System (PIAS Inc., Osaka, Japan). Migration area per unit length of the baseline (mm^2/mm) was compared among membranes. As a comparative scaffold of cell migration, a plastic cover slip (Celldesk®; CD, Sumitomo

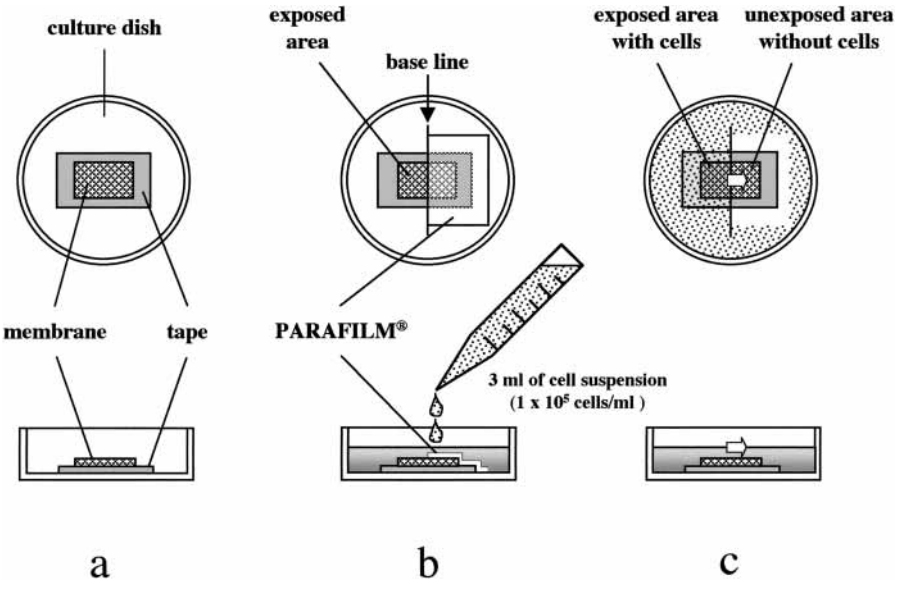

Fig. 1. Migration assay. a. A 3×5 mm section of the membrane was fixed to the bottom of a culture dish with sterile double-sided adhesive tape. b. Half of the membrane was closely covered by PARAFILM® (American National Can™) to leave an unexposed area for cell migration. The border between exposed and unexposed areas was marked as a baseline of cell migration. Membranes were then plated with 3 ml of cell suspension at an initial density of 1×10^5 cells/ml in α-MEM culture medium with 10% FBS and ascorbic acid. c. After 5 hours incubation, non-attached cells were completely washed out with PBS and the PARAFILM® cover was removed. After 3 days cultivation, specimens were fixed with 10% buffered formalin and stained briefly with hematoxylin for histometrical analyses.

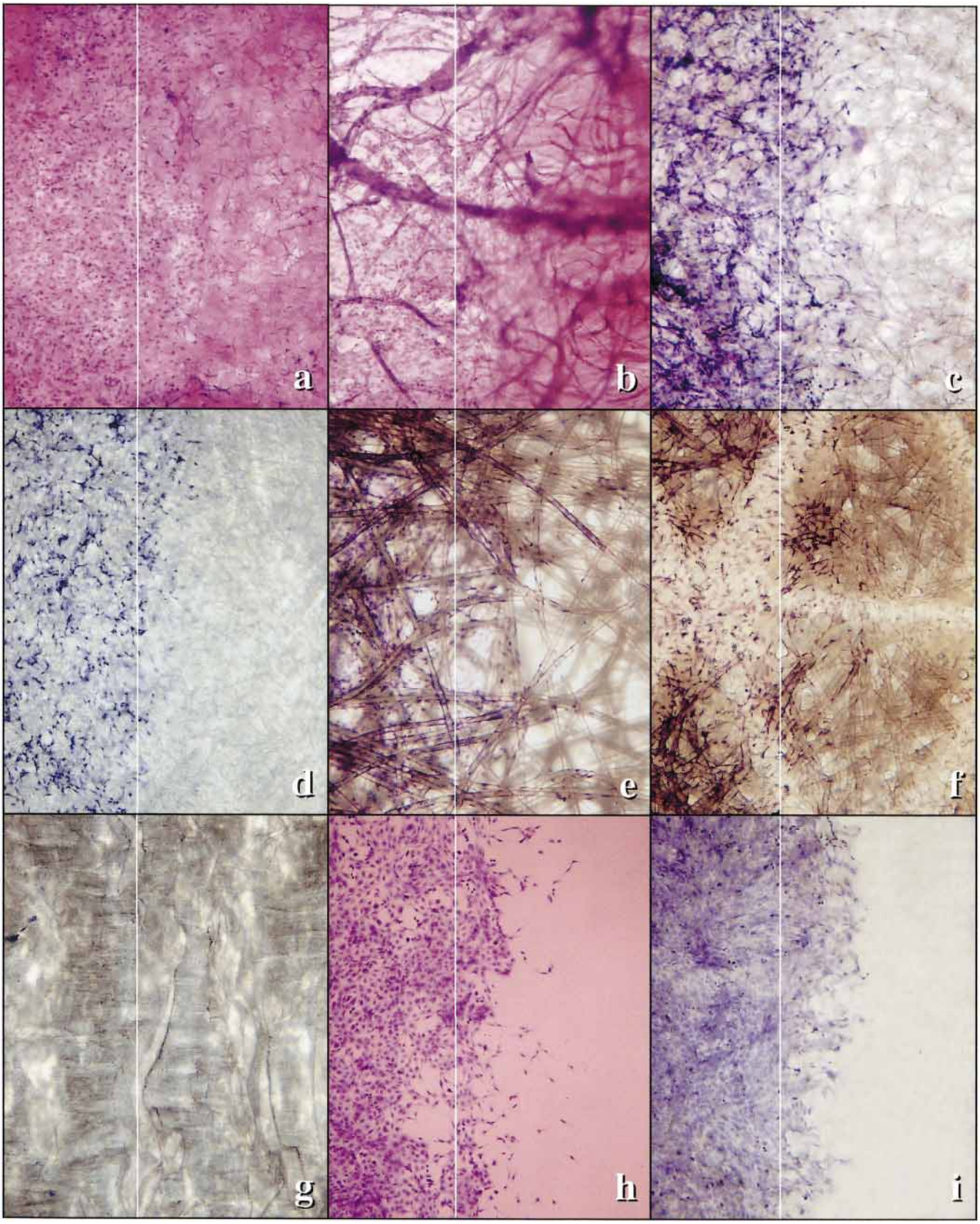

Fig. 2. Cell migration on the membranes examined. (a) bovine type I collagen (BioMend®), (b) porcine type I collagen (BioGide®), (c) bovine type I atelocollagen (Tissue Guide®), (d) polylactic acid (Epi-Guide®), (e) co-polymer of polylactic acid and polyglycolic acid (Resolute®), (f) co-polymer of polylactic acid and polyglycolic acid (Resolute XT®), (g) expanded polytetrafluoroethylene (Gore Tex®), (h) co-polymer of cellulose acetatenitrocellulose (Millipore filter®) and (i) a plastic culture cover slip (Celldesk®).

Bakelite, Tokyo, Japan) for cell culture was prepared for the migration assay in the same procedures. All samples were tested at least in triplicate.

Statistical analysis

The results were statistically analyzed using Wilcoxon's test for non-paired data. The P value at which statistical significance was inferred was chosen at $P<0.017$ ($0.05/\sqrt{9}$).

Results

Five hours after the cell seeding, the majority of MC3T3-E1 cells attached on the bottom of the culture dish and the membranes. Preliminary observation of the specimens at the start of cell migration showed a clear boundary at baseline between the unexposed fresh area and the exposed cell attached area (data not shown). Cells at this point were relatively rounded in shape and became more spindle-shaped with time. During the experimental period, there was no finding indicating a detrimental or otherwise influence of the glue from the double-sided adhesive tape.

At the end of the assay, cells migrated on the membranes to various extents (Fig. 2). Generally, cells migrated continuously from the exposed area to the unexposed area beyond baseline. Cell density of the migration area gradually decreased toward the front of migration. On the collagenous membranes (BM, BG and TG), cells seemed to migrate mainly along with fibrous structures of the membranes. However, the migration area on each collagenous membrane was different among the membranes with different fiber thickness and texture. On EG, cells migrated with the wall of honeycomb structures. On RL and RL-XT, cells migrated preferably along the fibers of the co-polymer. Only a small number of the cells attached to GT, and the net cell migration for this membrane could not be determined. On the smooth surface of MP and CD, rather uniform migration was seen. Fig. 3 is a bar graph of the cell migration on the membranes and CD. The results demonstrated that cell migration was greater in the order: RL>RL-XT, BM, TG, MP>EG, BG. Membranes except for BG, EG and GT showed the migration rate equal to or higher than CD on which cells generally grow favorably ($P<0.01$).

Discussion

The use of GBR has proved to be a suitable and fairly predictable technique for promoting bone regeneration (Hammerle 1999). A variety of membrane materials have been developed and employed in experimental and clinical studies for this purpose. These include PTFE, ePTFE, collagen, polyglactin 910, polylactic acid, polyglycolic acid, titanium mesh, calcium sulfate, and others. How factors such as membrane constituents, morphology, surface textures, size of perforations and duration of barrier function may influence GBR outcomes has not yet been completely understood. The data from this study demonstrate osteoblast affinity and migration when exposed to different GBR barrier membranes. For a barrier membrane to be successful, cell migration, attachment and proliferation to the materials is essential and for this to take place the material must have no deleterious effects on osteoblasts (Grinnell 1978; Burridge 1986; Burridge et al. 1987). Furthermore, it should also encourage cell spreading to a degree which promotes cell proliferation and migration. Cell culture systems serve as excellent models for examination of these events in relation to barrier materials.

Under the conditions of the present *in vitro* experiments, migration rates of osteoblastic cells on GBR membranes were different among the various membranes examined. Among the membranes tested, RL, RL-XT, BM, TG and MP showed migration rates equal to or higher than the culture dish, on which cells generally grow favorably. These findings are in agreement with our previous report that MP had the greatest amount of cells attached as compared to other membranes. BM, EG and RL showed a statistically significant higher osteoblast attachment when compared to Guidor and GT (Wang et al. 1997). This further supports the concept proposed by Burridge and his coworkers (1986, 1987), who suggested that inherent to cellular migration over a substrate (barrier) is the ability of cells to first attach to the substrate surface. Cell replication begins only after these events have occurred (Salonen & Persson 1990). Moreover, these results correspond well with the results of cellular proliferation on the membranes (Takata et al., 2001). We found that RL had the largest growth of periodontal ligament cells followed by TG. In addition, for both RL and TG it was noted that cells proliferated with

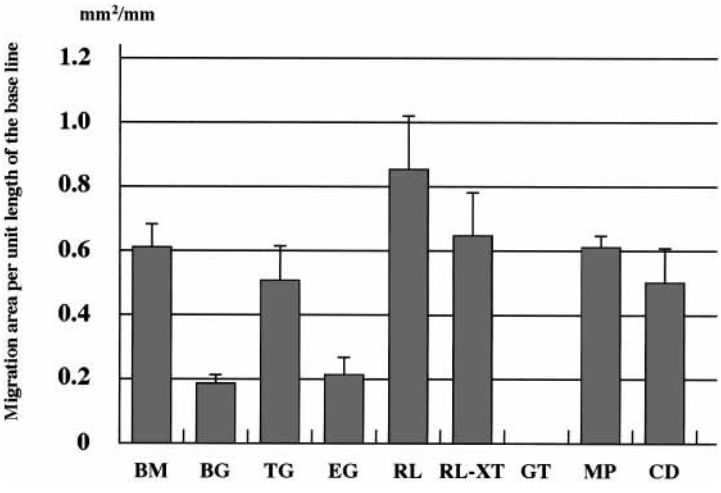

Fig. 3. A bar graph of the cell migration on the membranes and a plastic culture cover slip (Celldesk®: CD). BM: bovine type I collagen (BioMend®), BG: porcine type I collagen (BioGide®), TG: bovine type I atelocollagen (Tissue Guide®), EG: polylactic acid (Epi-Guide®), RL: co-polymer of polylactic acid and polyglycolic acid (Resolute®), RL-XT: co-polymer of polylactic acid and polyglycolic acid (Resolute XT®), GT: expanded polytetrafluoroethylene (Gore Tex®), MP: co-polymer of cellulose acetatenitrocellulose (Millipore filter®), CD: Celldesk®.

time, however with BM, EG and GT the cells remained at the same level after initial attachment. Cell clusters of alkaline phosphatase positive cells and foci of calcification were seen on all membranes except GT where a scant number of cells were seen.

From these observations, the GBR membranes examined here seem to provide a favorable scaffold for osteoblastic cells to proliferate and migrate. However, the differences noted in the migration rates of osteoblastic cells on these membranes may be attributed to the constituents, texture and other factors related to the membranes. RL and RL-XT are composed of a porous structure of glycolide polymer fiber and an occlusive membrane of glycolide and lactide co-polymer. It was a usual finding that cells attached on the fibers and migrated on them. MC3T3-E1 cells seemed to have a good affinity to the glycolide polymer. However, Simain-Sato et al. (1999) reported that fibroblasts cultured on RL showed rounded oval cells and cell fragments. A similar finding was also illustrated by Payne et al. (1996), who stated that fibroblasts cultured on the glycolide and lactide co-polymer showed abnormal flattened "fired egg" cell appearance and had a very limited number of cell attachments. The differences noted in these results can be explained in that two different cell types were used, Simain-Sato et al. (1999) and Payne et al. (1996) used gingival fibroblasts (from rat and human), whereas MC3T3-E1 osteoblasts were used in the present study. In addition, the acids released from these polymers may affect cell attachment, spreading, and migration and furthermore, influence the clinical outcomes as speculated by Hammerle (1999). However, we did not notice the effect of these acids in this experiment. Further study is definitely needed to clarify whether acid released from the degradation of lactide and glycolide co-polymers may influence cell culture environment as well as clinical healing/results.

EG is a polylactic membrane as is RL, although their modifiers are different; acetyl-tributylcitrate for EG and glycolide polymer for RL. These modifiers as well as the surface topography and spatial structure of the membranes may influence the different cellular responses. This is in agreement with Warrer et al. (1994), who suggested that cellular adhesion to solid surfaces is mediated by a protein film, and that the characteristics of this film depend on such properties as surface charge, chemistry and energy of the material.

Although BM, TG and BG are all collagenous membranes, BM and TG showed a high migration rate of cells but BG did not. Compared with the former two membranes, BG has a irregular structure and cells might be entrapped in the spaces among fine fibers of collagen. This may explain the different results observed in the present study. Nagahara et al. (1995) utilized a collagen membrane cultured with osteoblastic cells *in vitro* to promote calcification formation *in vivo* (Nagahara et al. 1995). The calcification was noted in the group treated with this complex at 28 days, while nothing was observed in the membrane alone group. In addition, Locci et al. (1997) also found that extracellular matrix, which consists primarily of collagen and chondroitin-4-sulphate, was the most suitable device to stimulate both cellular proliferation and extracellular macromolecule accumulation. These attempts imply that a collagen membrane in normal clinic use may promote formation of a thin osteoblastic cell layer to eventually enhance bone regeneration. However, further study is needed to confirm these hypotheses.

The migration rate of osteoblastic cells on GT could not be determined in the present study due to minimal cell attachment. This is in agreement with Salonen & Persson (1990), who also found significantly less migration of epithelial cells on ePTFE membranes as compared to MP. Similar findings were also reported by Simain-Sato et al. (1999), Locci et al. (1997) and Payne et al. (1996). The lack of adherence may be due to the decreased wettability, the surface roughness created by the overlapping fibrils, and/or the low protein-binding capacity (Brunette 1988; Salonen & Persson 1990). The advantages of minimal cell attachment of GT during GBR remain to be explored.

One must be cautious in interpreting results obtained from *in vitro* experimental models, since they can not recreate the complex interactions of cells *in vivo*. Therefore, the clinical significance of the data remains to be demonstrated. However, the data obtained from this study may promote development of an ideal barrier for guided bone regeneration.

Based on these preliminary *in vitro* results, it appears that RT-XT, MP, BM and TG promote osteoblastic cell migration better than other tested barriers. In addition, results from this study also suggest that GBR barrier materials *per se* may influence the process of bone regeneration *in vivo* through the effects of their presence on cell migration.

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Résumé

Pour évaluer les effets biologiques de matériaux barrière de la régénération tissulaire guidée (GBR) sur la migration de cellules ostéoblastes, la migration de cellules de souris ostéoprogénitrices (MC3T3-E1) a été étudiée *in vitro* sur différentes membranes. Huit membranes GBR proposées dans le commerce: collagène bovin type I, (BioMend®; BM), collagène porcin type I (BioGide®; BG), atelocollagène bovin type I (Tissue Guide®; TG), acide polylactique (Epi-Guide®; EG), co-polymère d'acide polylactique et acide polyglycolique (Resolute®, RL, ResolutXT®; RL-XT) téflon; e-PTFE (Gore Tex®; GT) et co-polymère d'acétate de cellulose et nitrocellulose (Millipore filter®; MP) ont été testées. Une partie de 3×5 mm de la membrane a été fixée au fond d'une boîte de culture à l'aide d'un adhésif double face et la moitié de la membrane a été recouverte par du PARAFILM® (American National Can™) afin de laisser une aire inexposée pour la migration cellulaire. La frontière entre les aires exposées et inexposées était indiquée comme départ de la migration cellulaire. Les membranes ont été mises en culture avec 3 ml de cellules en suspension à une densité initiale de 1×10^5 cellules/ml dans un milieu de culture alpha-MEM avec 10% FBS et de l'acide ascorbique. Après cinq heures d'incubation, les cellules non-attachées ont été complètement détachées avec le PBS et le recouvrement PARAFILM® a été enlevé. Après trois jours de culture, les spécimens ont été fixés à l'aide d'un tampon de 10% de formaline et brièvement colorés par l'hématoxyline. L'aire de migration cellulaire sur une membrane a été analysée en utilisant le système d'analyse d'images LA 500 et l'aire de migration par unité de longueur du départ (mm^2/mm) a été comparée aux autres membranes. Les résultats ont montré que la migration des cellules augmentait suivant l'ordre: RL>RL-XT, BM, TG, MP>EG, BG. Toutes les membranes sauf BG, EG et GT montraient

un taux égal ou supérieur de migration par rapport à une lame de recouvrement de culture en plastique (Celldesk®) (P<0.01) sur laquelle les cellules grandissent d'habitude favorablement. Seul un petit nombre de cellules se sont attachées au GT et la migration cellulaire nette n'a pû être déterminée. Ces résultats indiquent que les matériaux barrière GBR *per se* peuvent influencer le processus de régénération osseuse *in vivo* par leurs effets sur la migration cellulaire.

Zusammenfassung

Um den biologischen Effekt von GBR-Membranmaterialien auf die osteoblastische Zellmigration zu untersuchen, wurde die Migration von Osteoprogenitorzellen von Mäusen (MCT3T3-E1) auf verschiedenen Membranen *in vitro* getestet. Acht auf dem Markt erhältliche GBR-Membranen wurden getestet: bovines Typ 1 Kollagen (BioMend®; BM), porcines Typ 1 Kollagen (BioGuide®; BG), bovines Typ 1 Atelokollagen (Tissue Guide®; TG), Polimilchsäure (Epi-Guide®; EG), Kopolimer aus Polimilchsäure und Poliglykolsäure (Resolute®; RL, Resolut XT®; RL-XT), expandiertes Politetrafluoroethylen e-PTFE (Gore Tex®; GT) und Kopolimer aus Zelluloseacetat und Nitrocellulose (Milliporefilter®; MP). Ein 3×5 mm grosser Ausschnitt der Membranen wurde am Boden vom Kultivierplatten mit doppelseitigem Klebeband befestigt und die Hälfte der Membranen wurde dicht mit PARAFILM® (American National Can TM) abgedeckt, um eine nichtexponierte Fläche für die Zellimigration frei zu halten. Die Grenze zwischen der exponierten und nichtexponierten Region wurde als die Ausgangslinie für die Zellmigration definiert. Die Membranen wurden dann mit einer Zellsuspension mit einer initialen Dichte von 1×10^5 Zellen/ml in einem alpha-MEM Kulturmedium mit 10% FBS und Ascorbinsäure beschichtet. Nach einer Inkubationszeit von 5 Stunden wurden die nicht anhaftenden Zellen vollständig mit PBS ausgewaschen und die PARAFILM® Abdekkung wurde entfernt. Nach einer Kultivationszeit von 3 Tagen wurden die Proben mit 10% gepuffertem Formalin fixiert und kurz mit Hematoxylin gefärbt. Die Fläche der Zellmigration wurde mittels eines LA 500 Bildanalysesystems ausgewertet und die Migrationsfläche pro Einheitenlänge der Ausgangslinie (mm^2/mm) zwischen den Membranen verglichen. Die Resultate zeigten, dass die Zellmigration in der folgenden Reihenfolge abnahm: RL>RL-XT, BM, TG, MP>EG, BG. Mit Ausnahme von BG, EG und GT zeigten alle Membranen eine gleiche oder grössere Migrationsrate als ein Kulturabdeckglas aus Plastik (Celldesk®) (P<0.01), auf welchen die Zellen generell hervorragend wachsen. Nur eine kleine Anzahl von Zellen hafteten an GT und die Netto-Zellmigration für die Membran konnte nicht bestimmt werden. Die Resultate zeigen, dass GBR Membranmaterialien *per se* den Prozess der Knochenregeneration *in vivo* durch ihren Effekt auf die Zellmigration beinflussen können.

Resumen

Para evaluar los efectos biológicos de los materiales de barrera en GBR sobre la migración de células osteoblásticas, se examinó la migración de células osteoprogenitoras (MC3T3-E1) del ratón, *in vitro*, sobre varias membranas. Se probaron ocho membranas GBR comercialmente disponibles: colágeno bovino tipo I (BioMend®; BM), colágeno porcino tipo I (BioGuide®; BG), atelocolágeno bovino tipo I (Tissue Guide®; TG), ácido poliláctico (Epi-Guide®; EG), co-polímero del ácido poliláctico y ácido poliglícolico (Resolute®; RL, Resolut XT®; RL-XT) politetrafluoroetileno expandido: e-PTFE (Gore Tex®; GT) y co-polímero de acetato de celulosa y nitrocelulosa (Millipore filter®; MP). Se fijó al fondo de una placa de cultivo una sección de 3×5 mm de membrana por medio de una cinta adhesiva de doble cara, y la mitad de la membrana se cubrió de PARAFILM® (American National Can™) para dejar un área no expuesta para migración celular. El borde entra las áreas expuestas y no expuestas se marcó como linea basal de migración celular. Las membranas se cubrieron con 3 ml de suspensión celular con una densidad inicial de 1×10^5 células/ml en un medio de cultivo α-MEM con 10% FBS y ácido ascórbico. Después de una incubación de 5 horas, se lavaron completamente las células no adheridas con PBS y se retiró la cubierta de PARAFILM®. Tras 3 días de cultivo, los especímenes se fijaron con formalina tamponada al 10% y teñida brevemente con hematoxilina. El área de migración celular se analizó usando un Sistema de Análisis LA 500 y se comparó el área de migración por unidad de longitud de la línea basal (mm^2/mm) entre las diferentes membranas. Los resultados demostraron que la migración celular fue mayor en el orden: RL>RL-XT, BM, TG, MP>EG, BG. Todas las membranas excepto BG, EG y GT mostraron un índice de migración igual o mayor que el de una cubierta de plástico (Celldesk®) (P<0.01) en la cual las células tienen generalmente un crecimiento favorable. Solo un pequeño número de células unidas a GT y la migración de red de células de la membrana no pudo ser determinada. Estos resultados indican que los materiales de barrera para GBR, *per se* pueden influir en el proceso de regeneración ósea *in vivo* a través de los efectos de su presencia en la migración celular.

要旨

骨芽細胞の遊走に対するGBRバリア材料の生物学的効果を調べるために、様々なメンブレン上におけるマウスの骨芽細胞前駆細胞(MC3T3-E1)の遊走をインビトロで検査した。以下の8種類の市販GBRメンブレンを試験した：ウシ1型コラーゲン(BioMend®; BM)、ブタ1型コラーゲン(BioGide®; BG)、ウシ1型アテロコラーゲン(Tissue Guide®; TG)、ポリ乳酸(Epi-Guide®; EG)、ポリ乳酸とポリグリコール酸の共重合体(Resolute®; RL, Resolut XT®; RL-XT)、伸展ポリテトラフルオロエチレンe-PTFE(Gore Tex®; GT)、セルロース・アセテートとニトロセルロースの共重合体(Millipore filter®; ML)である。

3×5mmのメンブレン片を培養皿底面に両面テープで固定した後、メンブレンの半分をPARAFILM®(American national CanTM)で被覆し、細胞遊走のための非露出の領域を残した。露出領域と非露出領域の境界を、細胞遊走のベースラインとしてマークした。10%FBS及びアスコルビン酸を添加したα-MEM培地中に1×10^5細胞/ml濃度になるように調整した細胞懸濁液3mlをメンブレン上に播種した。培養5時間後、付着していない細胞をPBSで完全に洗い流し、PARAFILM®のカバーを取り除いた。培養3日後に、試料を10%緩衝ホルマリンで固定し、ヘマトキシリンで短時間染色した。メンブレン上の細胞遊走の領域は、LA500 Image Analysis Systemを用いて分析し、ベースラインの単位長さあたりの細胞遊走面積(mm^2/mm)を各メンブレン間で比較した。結果は、細胞遊走は次の順位に大きかった；RL>RL-XT>BM、TG、MP>EG、BG。BG、EG及びGT以外のメンブレンは、細胞が一般によく増殖するプラスチック製培養カバー・スリップ(Celldesk®)と同等あるいはそれより高い遊走率を示した(P<0.01)。ごく少数の細胞だけがGTに付着し、メンブレン上における細胞遊走は検出できなかった。これらの所見は、GBRバリヤー材料自体が、細胞遊走に対してその影響し、生体内において骨再生の過程に影響を及ぼしうることを示唆している。

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