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Detection of Th1 or Th2 cytokine profiles and immunolocalization of the cytokine secreting cells in the cellular immunity induced by the implantation of porcine dermal collagen membrane

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Keywords: Th1/Th2 cytokine profile; RT-PCR; ABC stain; PDCM

In 1992, we use gastric pepsin to remove the major antigenic determinant of a porcine dermal collagen membrane (PDCM) in order to improve the biocompatibility of this material. In one of pervious studies, we found the major cellular immunity induced by 3% glutaraldehyde (GA) conditioned PDCM was predominated by CD4+ T cells. The CD4+/CD8+ ratio was 2:1 when the material was implanted in muscular tissue 7 to 10 days. However, Mosmann and co-workers proposed a major subdivision of CD4+ helper T (Th)-cell clones based on differences in their pattern of cytokine production. The production of selected cytokines by local Th1- or Th2- type immune response to xenogenic graft may play a crucial role in the development of periodontal regeneration. Our proposal is to use cytokine specific RC-PCR to detect the existence of the relevant Th1 cytokine (e.g. IFN-γ and IL-2) and Th2 cytokine (e.g. IL-4, IL-10, and IL-13) 7-10-14 days after PDCM implantation. The cytokine secreting cells are also localized by using Avidin-Biodin Peroxidase Complex method (ABC method). 3% GA cross-linked PDCM and e-PTFE is intended to implant in

the dorsal skin of 20 S.D. rats. The shame operation is conducted on the one third of rats without grafting. The animals are enthanized 7, 10, and 14 days after the surgery.(n=6). The membranes and its adjacent tissues (2 mm thick) are harvested and homogenized for the detection of dominant cytokine -specific mRNA expression. The same operation and timetable are executed on the other 6 control S.D. rats in order to localized specific cytokine secreting cells with ABC method. Albeit various collagen membranes have been used in clinical experiments for GTR, few literatures indulge in the research of the cytokine profile induced by xenogenic membranes. Our study indicates that IL-2 profile of e-PTFE groups is similar to that of PDCM groups 7 days after implantation. However, the immunohistochemical stains of INF-γ, IL-4, IL-10, and IL-13 profiles of e-PTFE showed stronger intensities than those of PDCM groups. On the day 10th, only the immunol stain of INF-γ presents non-significant difference ($P<0.05$). On the day 14th, only the IL-10 profiles between two groups showed significant difference ($P<0.05$) It implies that PDCM may elicit a more trend of Th1 cells reactivity in cellular immunogenesis. On the other hand, e-PTFE may induce more trend of Th2 cells reaction. Therefore in guided tissue regeneration technique, PDCM is more suitable for the application in periodontal regeneration than e-PTFE as concern Seymour's hypothesis.

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ƒ ‡ PDCM „ guided bone regeneration(GBR)‟ ” ° ‟ «ª ‰¡s‡M/Š › /SPÐ€M¥ i · £ ¤†y ' † › M ' 'fl‰•Œ › A‟”¥ ´ † z ' ”¡¯¶¡/ • s'¡/Šƒ‰¥„3% GA cross-linked PDCM ' '‰Ífl ª l///] ¤†čell separation and space making ‟”Š ¶ ¿¡C

ƒ ‡ ¥iŠ Ì‹‟”‰ ƒ › † J ¥ ‰ /øfŠ»´B ¥Ł¿W/ Ø´¡.Aš‟x ¤ƒ¥Ð˝‟«¯ Ø ”'/ o‟”† M'Š K / ˇ ¥of† · ‟ –·¡M¶ ¥ Þ‚L¥H ‰ ƒ › ‡¥/Œ«˜‹ / _‟”† M'°¸ Ø†Ğ°Š K ‹ / ˇ†¥ «D –‘ƒ†›¡CŠ › ' »sŠ@‟”‰ ¥ ‰ ƒ › † J ¥‰™”‰ø ƒ”› Š‡ ¥ ¶fH˜/Œ« ‹ ‰ ƒ ›†¡Ð¥ ' ¥... ¤ ‟”^ { Š †¥Šƒ ¶q¡A ªs¥ » ‰ ƒ › † ¥ ‰/øº'Š † ›MŠ K ‹ / ˇ † ‹ Œ»„Œ€ ƒ«Ì„Ì Š › · ¿ ¿¤anti-rat CD4, anti-rat CD8, /¸anti-rat CD11b ¥ † fiŁŠØ¡AŠQ¥¸Avidin-Biotin Peroxidase Complex Method(ABC Method)¡ A / ‡P/Šfi ¶¡˘ ‚ 3% cross-linked PDCM' † ´·// ' / o/Scellular immunogenecity,¤ ¶i/@¤ B ˜ »2% GA cross-linked PDCM' »/ o/ Š † ›MŠ K / ˇ†//T cells»Þmacrophages¥ifl æ”‡Š¤ ƒ ,¤^, „ ˇÞP‚PDCM' † ´·/Š Ì‹¥ifl /Š ´ ²¡C † ‟ G ¯ ¥ ¥» Œ¯CD4¡ÞCD8 › p˜‟” ”G¡AŠ › ¥i¥H ф{ ª› ‟ flg/ ˇ†/]¥HCD4⁺T† ›M¥D ¥M¡M' †˜/ Ć »P†˜/ Ø fi † ›M..˜¥ „ F ¤˜‚Mfp¥ÞCD4:CD8/ æv¥ – ‟æ2:1¡CPDCM' / _/ Š † ›M'° Š K ‹ /ˇ¥HCD4⁺ T † ›M¥D ¡Af ¥Þ¥ ‹ ¡D/Š¥ ¤‰† ›M¥¡ °‹ ¤ ,¤ ' / _/ŠŠ K ‹ /ˇ¥ifl /æ‚ß ^ ƒ type IV cell-mediated immune reaction¡C

Mƒ ¥ Š › ¥ «e/Šx ¡¯Ø‚ ¯ ”G¥i‟ ¡G¥ » ‰ ƒ › †J‚¥m‰Ƒ/¥j „«¯ Ø”/ T ¶j«‚‚M¥ 1 † ´·// o †{/j¶q¥ ¤‰† M/»Š ¶ ¡Aƒ ¥ Š K ‹ /Š˜[´I, ˜ ¡ Æ„¥ ¤‰† M¥fl ‹ OŠŒ”tŠ › Šet{† ›M ¡]Antigen presenting cell, APC¡^‟”¤ ƒ ¡A† ¤·¥ ¤‰† M¥fl •|˜ ' æ ¥ ŒY¤·›M¿ fl ¡]cytokines¡^¤^– ‰~¤ Š › ‰/Ƒ‰ , ‘«Æ»P¥»¤ ›† ›M‟ ›–/Š¥D›n† ´·‹ fie'°‰ ƒX¯Ø¡]major histocompatibility complex, MHC¡^ †ƒX¡AŠ ƒ¤MHC-peptide †ƒX¯Ø¡Mƒ@ƒPŠe†{ „T† ›M‟ ›–”– ¤ „¡Mƒ ¥ fl Šw/ŠT† ›M¡]ƒpCD4⁺»ÞCD8⁺T† ›M¡^' ¿ ° ¡M¶if / o/@† ‡ŒŠ K ¤ ‰ / ˇ†. ƒ] ƒ ‚/ F 3% GA-PDCM¥ifl ' ¶ ¸ £´ƒ¡X† ˜7/ ¤ †˜10/ / _/æ‚ß'œ¯ª/ŠCD4+/CD8+ T cells phenotype activity¥H¥~, Š › /]Š ˘ ¥ 3% GA conditioned PDCM ' / o/ŠT-cell reaction /Š cytokine profile, ¶i/@¤B/ F 3% GA-PDCM' / _/ Š † ›MŠ K ‹ /ˇ/Š¶if Łƒ¡VA¤s˜ ¥HTh1 cells‹ ƒV' ¸¥HTh2 cells‹ ƒV¡A¥H«K¶i/@¤B/ ˜ ¨¥X ª› PDCM' † ´·/† ‚ , ¥ifl /Š ´ .

¥ « e ¥ ' guided tissue regeneration ⁄ § ⁄ ˜ m ⁄ ⁄ ˆ¥ biodegradable collagen membrane
‡ Q † ´ · § l ƒ ‹ " ⁄ ‡§ §K Q ƒ]¤ƒ „ § ˘ – ¥ » ‹ ª ¤ s › p ˝¥ ¡ · E ¤ " ˙ ƒ N ‹ ‹

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Table1.†˜7⁄ fi ePTFE »P PDCM A¡B¡C ˘cytokine § K ‹ V ƒ § e ƒ–j« ›¨ (Mean±SD)

		INF-r	IL-2	IL-4	IL-10	IL-13
A	PDCM	2.75±0.5	2±0	2±0	1.5±0.6	0.25±0.5
	ePTFE	3±0	2±0	3±0	2.75±0.5	1.5±0.6
B	PDCM	3±0	2±0	1±0	0.75±0.5	0±0
	ePTFE	3±0	2±0	3±0	3±0	1.5±0.6
C	PDCM	2.25±0.5	1.25±0.5	0.75±0.5	0.5±0.6	0.25±0.5
	ePTFE	3±0	1±0	3±0	2±0	1.25±0.5
Total	PDCM	2.67±0.5*	1.75±0.5	1.3±0.6*	1.0±0.7*	0.2±0.4*
	ePTFE	3±0	1.7±0.5	3±0	2.6±0.5	1.4±0.5

Immuno Stain Intensity Ranking Test (P>0.05)

0:No stain

1:Slightly stain

2:moderate stain

3:strongly stain

Table2.†˜10⁄ fi ePTFE »P PDCM A¡B¡C ˘cytokine § K ‹ V ƒ § e ƒ–j« ›¨ (Mean±SD)

		INF-r	IL-2	IL-4	IL-10	IL-13
A	PDCM	1.5±0.6	0.75±0.5	1.5±0.6	1.75±0.5	0.75±0.5
	ePTFE	2.25±0.5	2±0	2.25±0.5	2.75±0.5	1.25±0.5
B	PDCM	1.75±0.5	1.25±0.5	2±0	2±0	1±0
	ePTFE	1.75±0.5	1.75±0.5	2.25±0.5	2.75±0.5	1.75±0.5
C	PDCM	1.5±0.6	1.25±0.5	1±0	1±0	1±0
	ePTFE	1.5±0.6	1.25±0.5	2±0	2±0	1.25±0.5
Total	PDCM	1.6±0.5	1.1±0.5*	1.5±0.5*	1.6±0.5*	1.0±0.3*
	ePTFE	1.8±0.6	1.7±0.5	2.2±0.4	2.5±0.5	1.4±0.5

Immuno Stain Intensity Ranking Test (P<0.05)

0:No stain

1:Slightly stain

2:moderate stain

3:strongly stain

Table3.†˜14 ⁄ fi ePTFE »PPDCM A¡BB¡BC ˘cytokine § K ‹ ‹ V ƒ §ƒ(Mean±SD)

		INF-r	IL-2	IL-4	IL-10	IL-13
A	PDCM	1.25±0.5	2±0	2±0	2±0	1±0
	ePTFE	2.5±0.6	1±0	1.5±0.6	2.75±0.5	1±0
B	PDCM	1.5±0.6	1.5±0.6	1.75±0.5	1±0	1±0
	ePTFE	2.75±0.5	1.5±0.6	2.25±0.5	1.75±0.5	1.25±0.5
C	PDCM	2±0	0.75±0.5	1±0	1±0	1±0
	ePTFE	2±0	1±0	2±0	1.75±0.5	1±0
Total	PDCM	1.4±0.5	1.4±0.7	1.6±0.5	1.3±0.5*	1.0±0
	ePTFE	2.4±0.5	1.2±0.4	1.9±0.5	2.1±0.7	1.1±0.3

Immuno Stain Intensity Ranking Test (P<0.05)

0:No stain

1:Slightly stain

2:moderate stain

3:strongly stain

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¥ «¥Mosmann[3], Bloom[4],Seymour[5] ¥ ƒ h ƒ ˙ ˝¤ ⁄ §§» ª{ ‹ ¡MCD 4+MPCD8+T† › M ¤ ƒ‡⁄£ƒP ⁄§† › M ˝ →–¡Mƒ ,, O ƒfl§§–w¡ §⁄¡M¤ ª ƒ ‡ ⁄ £ ƒ P ⁄§T§† ›M¥ ¥ i ˜¥ƒXR⁄§† ›M¿E fl ¡M‰ , ˝§ K ¥ ƒl ¡Mƒ] ƒ „ T† ›M⁄§¥ ƒl ' ° ⁄^¡M§ ^ H ¥i¤ † › M ˝ →–¤ ¤ ¿„T† ›M ⁄§˝ † { « ¥H¡¥M† { ⁄⁄¥Si¤ CD4+»PCD8+T† ›M' † ´ ⁄⁄' ˝c⁄§fl S ' w ¿›Mfl (cytokine)¡Mƒ –NT† ›M⁄§¥ ƒl ˘⁄ ‹ †˜⁄@«‹(Th1)⁄ ¸†˜⁄G«‹(Th2)T† ›M⁄ ˘‡¡ @ ⁄⁄†˜⁄@«‹CD4+T† ›M⁄§ APC(antigen presenting cells)‹ O¥ ¥ ¤‰† ›M' ¸B† ›M' ¥ ¥q¡M¤^' § K ‰ , ˘⁄¥ Th1 cells˜ ¥X IL-2 ¡BINF-r ¥† ›M¿ fl ¡ A ¶ i ƒ§B¤† ›M' ¸⁄ ⁄ ‹« „ L ⁄ ˘‡¡] delayed-type hypersensitivity¡^ ¥§ K ‰ , ˘⁄ ˘‡¡Qƒ„⁄@‡ ƒ§C⁄§§ ¥ ¸' ˙† zW¡M¥ fil ' ‰ w œ'P ´·˝ ˝ fl^¡M ˘ ' ¸‡ ‡yƒ‡§Q' ⁄ dP† ´··_› ¶iƒ ƒ A ¥ ˝ ˝ ¡C„ƒ ' Th2 cells«h¥i¿ ° ¥D›n¥ B† ›M' ¥ ⁄§ APC' §e†{⁄§MHC-peptide † X¯ØA¤^˜ 'æIL-4¡BIL-10 ¥† ›M¿ fl ¡Fƒ ¤ ‡ † § ¥ ¸¥] ‹ A B§U † ›M¡B§ ¤ DTH¡A †˝G–N«P¤˘B† M§ƒ¸¤fl §w⁄§§ ¯Ø⁄ ¸˜ ¥Xfl §w⁄§† ›M¿ fl ¡]ƒp¡GIL-1¡¡^Aƒ « ø ˜ ¥[⁄‡dP† ´·˝ ˝ fl^¡C

ƒ ƒ„¥ ¥ » C¯⁄§cytokine profile⁄§⁄ æ , ß ª¡¥APDCM' ¥ ⁄U¶ ¸ ⁄ ƒ ›·§† ›M' °§ K ⁄ ˘‡, ß` ƒV Th1 cells reaction¡Gƒ e-PTFE«h, ß⁄ ‡..ƒVTh2 cell reaction¡C˝ ⁄W' ›z¡A¤^¤ Seymour et al. ⁄§ †z‰ ¡A§ › » ‹{ PDCM⁄æe-PTFE§ A¥ ¸' ⁄ dP ⁄ ƒ A˝⁄ ‡N¡A¥H„ F ⁄ œ ' ƒA¥§¥ ...–¡C

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¥ » ‡ ł § i ¥] ‹ A § K ‹ ⁄ ˘ ˙ ¡† A´ § ‹ Vƒ› ⁄ § ‡ ¡ ¥ p ⁄ ¤ ¿ ¡ ¡ § ⁄ § ¡ A ‹ fl } Jagoe et al. (Histochem J, 1991, 23:541-7) ⁄ § § ‡ N ¡ ˆ A ˘ ¥ D ˘ [‹ « ¡ A ˘ [£ ¥ „ PDCM ' GTR technique ⁄ W ⁄ § • s ˆ ¡ A § ˘ – ¥ … ¤ fl ˙ ‹ ¤ ¤ ƒ ⁄ ł § « – – ¡ C ƒ ˆ RT-PCR ‡ ¥ « h « ˜ ¡ Q g ¡ Q ‰ § « ¥ ¥ fl ` ¥ † ¶ ¥ W Western blot, – j ¡ A ¥ H ƒ U ″ cytokine ⁄ § antibody ¥ ‰ » INF » PIL ⁄ § ƒ ƒ b ¡ C