出國報告

類別：國際研討會

參加 2013 年美國組織庫年會心得報告

服務機關：台北榮總兒童心臟科

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摘要（含關鍵字）

美國組織庫協會(AATB) 2013 年主題是組織庫的品質管理和器官勸募，組織工程與再生醫學的發展。由本次會議的內容可知美國在組織庫領域有非常蓬勃的發展，由人體組織經由組織工程結合幹細胞科技是未來的再生醫學的趨勢。本人是美國組織庫協會的會員及認證通過的臨床專家(Certified Tissue Bank Specialist 簡稱 CTBS)，並自 2013 年 2 月起，被美國組織庫協會的科學技術委員會(Science and Technology Committee 簡稱 STAC)聘任為委員，今年前往參加年會除了學習新知外，主要的目的是參加 STAC 委員會的年度會議並發表二篇論文。

1. 組織庫 2. 再生醫學

一、目的

榮總心臟瓣膜與血管組織庫的所有作業規範皆須核合美國組織庫協會(AATB)與美國食品藥物管理局(FDA) 的規定，本院心臟瓣膜組織庫與國際接軌，操作必須須符合全球組織庫學會規定的標準作業流程，本人是美國組織庫協會的會員及 2012 年認證通過的臨床專家(Certified Tissue Bank Specialist)，並自 2013 年 2 月起，被美國組織庫協會的科學技術委員會(Science and Technology Committee 簡稱 STAC)聘任為委員，今年前往參加年會除了學習新知外，主要的目的是參加 STAC 委員會的年度會議並發表二篇論文。

二、 過程

本次會議是在美國華盛頓市的 National Harbor 國際會議中心舉行。National Harbor 國際會議中心風景非常優美，隔著河和美國國父華盛頓的故鄉 Alexandra 鎮相對望。大會有安排到此鎮遊覽，Alexandra 鎮是美國第 2 老的城鎮，建築的風格基本上是非常英式的城鎮，古董店名牌店和高檔美食餐廳林立(今年美國波士頓龍蝦盛產)，到處充滿人潮，在這個美麗鎮城上可以感受到富足的美國，我們會議的期間歐巴馬總統宣佈公務員放無薪假，據說很多政府單位真的關閉了，我們這些遊客並無感受，但是美國人好像見面都只談這個話題，連大會晚宴的脫口秀也在取笑這件事，不知道台灣未來會不會也和美國一樣，政府窮到要放無薪假。

三、 心得

美國組織庫年會是全世界一年一度的組織庫大拜拜，所以場面非常浩大。今年的大會 Jeanne Mowe Memorial Lecture 的題目是:Vascularized Composite Tissue Allografts，這是由一位病人開場描述她的奮鬥過程，她 17 歲得到突然因嚴重的感染昏迷及休克了 8 天，救醒後四肢缺氧壞死，經過各種高壓氧療無效後先切除雙下肢

(beneath knees) ，雙手肘以下繼續治療無效後也切除。他的下肢裝上了義肢，上肢接受了雙上肢的組織移植(第一個成功的案例)，這是一個無數次手術的治療，其是結締組織的去除過程與辛苦的復健令人非常的感動她的意志力和毅力，最重要是她並希望人生因此變成黑白，他最後開始畫畫，而且堅持要搬出父母家而獨居，過程可以看到美國醫療不只在治療部份的專業，更在多科部之間的配合，她醫接著講述手術的方法，和組織庫如何配合治療，提供多次的上肢組織及及神經來供外科醫師完成手術。

本次另一個非常精采的演講是由衛生組織的專家 Dr. Lu Noe 演講 The emergency of global government for medical products of human organ，以前我並不知道原來整個人類同種組織的移植(包括器官) 的全球管理是如此重要的議題，WHO 有 6 個辦公室進行這個議題的推廣，亞洲的辦公室設在菲律賓(WPRO) ，有關組織及器官捐贈的監控是非常重要的，因為這些稀有的醫材可以變成非常昂貴的商品，在貧窮的國家就變成了有錢國家的人肉市場，來路不明的組織供應者在這個世界上非常猖狂，一個法國的組織庫被查獲是非法來自南歐的國家，德國二年前也出現醜聞，導致捐贈者少了 30%，沒有提到大陸的情形，當然疾病感控也是他們的重點，美國有一位組織捐贈者是 HIV window 期的組織，他的骨粉及骨骼製品捐了 1000 多人。

另外開幕當天竟這有一個主講的講題為 What happen when the government shunts down? 主要是 FDA 官員來強調如果沒有 FDA 管控美國的 medical device 及 tissue product 會變成怎樣，是個非常有”梗”的演說， 把 medical device 及 tissue product 的管理說的非常淺而易懂，大家都紛紛發言支持 FDA，表達國會亂搞真的很不應該，如此看來美國的國會和台灣的立法院在民眾的眼中也是非常相似的。

今年前往參加年會除了學習新知外，主要的目的是參加 STAC 委員會的年度會議委員，以住我參加會議都會主動的尋求和專家會談的機會，而這些人(如 Cryolife 公司負責心臟血管研發的主管 Steven Goldstein，Cryolife 公司是世界最大的心瓣

膜血管公司，Lifenet 公司主管心臟瓣膜及血管研發的 Alyce Linthurst Jones 博士，Lifenet 公司是世界第二大的心瓣膜血管公司，Georgia Tech 的 Kelvin Brockbank 教授，他是目前 AATB 心臟血管研發委員會(STAC)的主席，他的專長是處理血管，)都是 STAC 的委員，這次有機會這些全美最有名的專家共同開會，覺得非常愉快與受用良多，開會期間我有多次和他們請益的機會。

會前委員會的秘書一直要我提研究計劃，以往我沒擔任美國協會的委員的經驗，覺得菜鳥委員還是保守一點去當聽眾就好，到了才知道原來這個研發委員會的 15 位委員都是美國現今很重要的科學家，這些委員每人個別或共同可以於會前提一個研究計劃上限 100 萬美金，由委員內審通過後送理監事會通過，上述 Kelvin 及 Steven 都個別提了一個 30 及 50 萬美金(二年)的研究計劃，Alyce 更是提足了 100 萬美金(三年)研究計劃，會後理事長還說我們委員不夠踴躍總共只提了 300 多萬美金的計劃，他就下週全數送給理監事會認可，可惜不能補申請，大家都很關心我這個外國菜鳥委員，鼓勵我明年再提，這讓我對美國協會研究經費的額度算是開眼界了。

AATB 年會都是邀請演講，只有最好的三篇 Free paper 可以口頭報告，並且提供免付年會會費的獎勵，其他論文都是壁報論文，今年我們團隊發表了二篇都是壁報論文，都是關於小口徑幹細胞血管的研究，一篇是方法另一篇是電子顯微鏡觀察的結果(請見附件)，明年再努力。

四、 建議事項（包括改進作法）

本院組織庫已於 100 年 5 月 19 日通過 TFDA 的查核，應在這個基礎上再做進一步的努力，今年回國後已於 103 年 10 月 8 日於本院心臟外科晨會提出專題演講，希望能在未來臨床使用上列為工作的重點，提升本院心臟治療的水準，。

Sphingosine 1-phosphate Potentiated Endothelial Cell Attachment on Decellularized Human Umbilical Vein as a Scaffold for Vascular Tissue

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BACKGROUND

Human umbilical vein (UV) has been reported as a potential scaffold for vascular tissue engineering. As an allogeneic vessel, the cellular components that induce immunoreaction should be removed. However, the lack of the endothelial cells denotes the loss of the anti-thrombogenic function. Therefore, re-cellularization is a necessary procedure for a replacement vascular endothelial cell. Sphingosine-1-phosphate (S1P), a low molecular-weight zwitterionic lysophospholipid, is an important cellular metabolite that regulates several diverse cellular functions, including cell survival, proliferation, differentiation, motility, apoptosis, calcium homeostasis and adhesion.

AIM

- **This study focuses on building a novel recellularization condition with S1P for developing the human umbilical vein as a functional scaffold for vascular tissue engineering.**

METHODS

1. Preparation of decellularized UV:

UVs were incubated with 0.1% SDS buffer and then with fetal bovine serum containing medium for 2 days to reduce the residual cells.

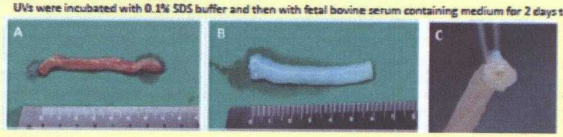

Figure 1. The decellurization of human umbilical vein.
A, Fresh human umbilical vein ;
B, Decellularized human umbilical vein;
C, Correlated cross-section.

2. Recellularization

Human umbilical vein endothelial cells(HUVECs) and endothelial progenitor cells (EPCs) were stained with Cell TrackerTM Green before experiment. The UVs were seeded with HUVECs or EPCs in static and rotational seeding conditions.

Static cells seeding: decellularized UV segments were cut into open patches and seeded with 4x105 cells/ml of Cell TrackerTM Green labeled. HUVECs or EPC and 1mL of EGM-2 were added to each well, EGM-2 was premix with 1uM S1P before added. Cells were then incubated for 24 hours at 37℃ and 5% CO_2.

Rotational cells seeding: Decellularized UV segments were inverted inside (luminal surface) out and the veins were fixed in the middle of the tube by a glass rod. Cell TrackerTMGreen labled cells were suspension in the culture medium with 1 uM S1P. The culture tube were rotated around the central axis and the rotation was performed for 24hours at 1 rpm at 37℃.

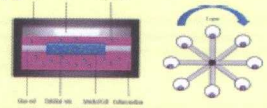

Figure 2. The rotational cell seeding profile. *Left*, The human umbilical vein was inverted inside out and fixed in the middle of the tube. The endothelial cells were suspended in the culture medium. *Right*, The culture tube were rotated around the central axis and the rotation was performed at 1 rpm at 37℃.

3. The attached cells on the UV were visualized by fluorescence microscopy at 517 nm. Ten fields were chosen randomly to count the number of the cells by Metamorph software. Neo-epithelial layers of cellularized UV were played HE stain and stained with DAPI, CD31 and CD34 marker. Data was analyzed with ANOVA test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

1. The quantitative data indicated that the S1P stimulated significantly the HUVECs and EPC cells attachment on the umbilical veins by different seeding methods in 24 hours (Figure 3).
2. The morphologic data of HE stain suggested that SIP could enhance the recellularization of HUVECs and EPCs. (Figure 4).
3. After 24 hours in culture, CD31 marker can be well demonstrated in neo-epithelial layer seeded by HUVECs and EPCs. CD34 marker can be demonstrated in neo-epithelial layer seeded by EPCs only. Using CD31 and CD34, we confirmed that the HUVECs and EPC are seeded to the inner layer of decellularized umbilical vein. Compared with the DAPI result, S1P did not significantly decrease the expression of CD31 expression in both HUVEC and EPC. Nevertheless, S1P decreased the expression of CD34 expression of EPC significantly (Figure 5 and 6).

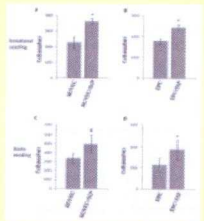

Figure 3. Number of cells seeded on the decellurized UV counting by MetaMorph. A, B, Numbers of HUVECs and EPCs by rotational seeding method. C,D, Numbers of HUVECs and EPCs by static seeding method. Data was shown as mean ± SD. * $p < 0.05$ indicate a statistically significant difference.

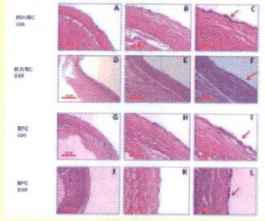

Figure 4 HE stain of HUVEC and EPC seeded to the inner wall of decellularized umbilical vein . A, Seeding HUVEC only; B, Seeding HUVEC treated with S1P; C, Seeding EPC only and D, Seeding EPC treated with SIP were well seeded (red arrow) on the inner wall of decellularized human umbilical vein. Original magnification, 200X; Scale bar of A, D, G, J = 10 um, Scale bar of B, E, H, K = 25 um, Scale bar of C, F, I, L = 50 um

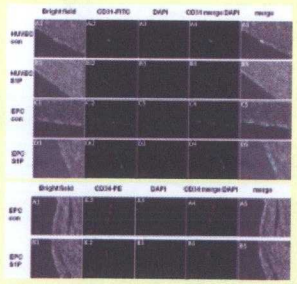

Figure 5. S1P enhanced HUVEC and EPC cell adhesion to inner vascular wall of decellularized human umbilical vein. The neo-endothelial layer (*A1, B1, C1 and D1*) were seeded with HUVEC or EPC. Both the HUVEC (*A2, B2*) and EPC (*C2, D2*) were attached on the inner vascular wall. Merged images of CD31 (*A2, B2*) and DAPI (*A3, B3*) showed that neo-epithelial layer were seeded with HUVEC (*A4, A5*) and EPC (*B4, B5*). SIP can enhance the HUVEC (*A2 , B2*) and EPC cell (*C2, D2*) adhesion to inner vascular wall. Compared with the DAPI result, SIP did not decrease the expression of CD31 expression in both HUVEC (*A4, A5*) and EPC (*B4, B5*). Original magnification, 200X; scale bar = 50 um.

Figure 6. S1P enhanced EPC cell adhesion to inner vascular wall of decellularized human umbilical vein. CD34marker was used to ensure that these neo-endothelial layer (*A1, B1*) were seeded with EPC. Merged images of CD34 (*A2, B2*) and DAPI (*A3, B3*)) suggest EPC were attached on the inner wall. SIP can enhance the EPC (*A4, A5*) and (*B4, B5*).

CONCLUSION

HUVECs and EPCs cells that treated with S1P attached better than the cells without S1P treatment, this suggested that S1P may be a good factor stimulating the endothelial cells adhesion on decellularized umbilical vein.

LITERATURE

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