充生，中山泰介，吉田恭史，松岡祐貴，木下　肇，佐田政隆，北川哲也
エゼチミブ投与が動脈リモデリング・機能へ与える影響の検討
第 42 回日本血管外科学会学術総会（青森県），2014 年 5 月 21 日 - 23 日

14. 木下　肇，中山泰介，菅野幹雄，黒部裕嗣，神原　保，藤本鋭貴，北市　隆，北川哲也
感染性腹部大動脈瘤に対して上腸間膜動脈に snorkeling EVAR を施行した 1 例
第 42 回日本血管外科学会学術総会（青森県），2014 年 5 月 21 日 - 23 日

15. H. KUROBE, T. Motoki, Y. Hirata, M. Sugano, T. Nakayama, H. Kinoshita, T. Kanbara, E.Fujimoto, T. Kitaichi, T. Hori, H. Sogabe, M. Sata, T. Kitagawa.
PPAR-γ Agonist Administration Attenuates inflammation In Patients With Aortic Aneurysm
AATS Aortic Symposium 2014（New York, NY, USA），April 24 - 25,2014

16. Hajime kinoshita, Taisuke Nakayama, Mikio Sugano, Hirotsugu Kurobe, Tamotsu Kanbara,Eiki Fujimoto,Takashi Kitaichi ,Tetsuya Kitagawa
The efficacy of endovascular treatment for type B aortic dissection
AATS Aortic Symposium 2014（New York, NY, USA），April 24 - 25,2014

17. 北市　隆，木下　肇，中山泰介，菅野幹雄，黒部裕嗣，神原　保，藤本鋭貴，北川哲也
特異な臨床経過を認めた静脈血栓塞栓症の 2 例
第34回日本静脈学会総会（沖縄県），2014年4月17日 - 18日）

18. 木下　肇，中山泰介，菅野幹雄，黒部裕嗣，神原　保，藤本鋭貴，北市　隆，北川哲也
うっ滞性皮膚潰瘍を合併した下肢静脈瘤に対して血管内レーザー焼灼術の有効性
第 34 回日本静脈学会総会（沖縄県），2014 年 4 月 17 日 - 18 日

19. Hirotsugu Kurobe, Noriko Sugasawa, Yoichiro Hirata, Mitsuo Shimabukuro, Taisuke Nakayama, Takeshi Yoshida, Mark W. Maxfield, Tetsuya Kitagawa
Vascular remodeling effects by administrating Ezetimibe after arterial wire-injury in mice.
ASCVTS 2014 (ISTANBUL,TURKEY), April 3 - 6,2014

20. 藤本鋭貴，筑後文雄，中山泰介，木下　肇，菅野幹雄，黒部裕嗣，神原　保，割石精一郎，加納正志，北市　隆，北川哲也
胸部大動脈破裂に対する緊急TEVARの検討
第 114 回日本外科学会定期学術集会（京都府），2014 年 4 月 3 日 - 5 日

H．知的財産権の出願・登録状況
（予定を含む。）

1．特許取得

なし

2．実用新案登録

なし

3.その他

1．PCT/JP2014/06317
平成26年4月22日PCT出願
2．特願2015-038961
平成27年2月27日出願
3．特願2014/234767
平成26年11月19日出願
4．2015年7月までに1件出願予定

Ⅲ. 学会等発表実績

学 会 等 発 表 実 績

委託業務題目「生体内分解性素材を用いた国産治療デバイスの開発
-経カテーテル的心房中隔欠損孔閉鎖デバイスの開発-」
機関名　徳島大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
生体吸収性素材を鋳型とした再生血管移植の研究と臨床の現状	黒部裕嗣 日比野成俊 太良修平 杉浦唯久 Christpher K. Breuer 新岡俊治	第45日本心臓血管外科学会学術総会（京都府）	2015年2月16日 - 18	国内
当科における破裂性腹部大動脈瘤に対する緊急ステントグラフト内挿術の検討	木下　肇 藤本鋭貴 菅野幹雄 黒部裕嗣 神原　保 割石精一郎 加納正志 筑後文雄 北市　隆 北川哲也	第45日本心臓血管外科学会学術総会（京都府）	2015年2月16日 - 18	国内
活性型第X凝固因子によるマクロファージ活性化を介した新しい動脈硬化進展機序の検討	原　知也 福田大受 田中君枝 東邦康智 平田陽一郎 八木秀介 山田博胤 添木　武 若槻哲三 島袋充生 佐田政隆	第44回日本心脈管作動物質学会（高松市）	2015年2月6日-7日	国内
肺高血圧症モデルマウスにおける血管構築細胞の低酸素応答転写因子の役割	井口道代 冨田紀子 今西正樹 黒部裕嗣 菅澤典子 佐藤　至 松永慎司 冨田修平	第67回日本薬理学学会西南部会(福岡県)	2014年11月23日	国内
小児開心術後縦隔炎に対するVAC療法と閉胸のタイミングについて	北市　隆 木下　肇 黒部裕嗣 神原　保 藤本鋭貴 阪田美穂 早渕康信 北川哲也	第10回四国小児循環器病研究会(愛媛県)	2014年11月15日	国内

当科における破裂性腹部大動脈瘤に対する緊急ステントグラフト内挿術の検討	木下　肇 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 加納正志 北市　隆 筑後文雄 北川哲也	第55回日本脈管学会総会(岡山県)	2014年10月30日 - 11月1日	国内
下肢動脈血栓性閉塞に対し血栓除去とEVTが有効であった1例	木下　肇 藤本鋭貴 黒部裕嗣 神原　保 北市　隆 北川哲也	第14回血管外科アカデミー(徳島県)	2014年9月6日	国内
下肢動脈閉塞に対するハイブリッド治療の経験	木下　肇 黒部裕嗣 神原　保 藤本鋭貴 北市　隆 北川哲也	第89回中国四国外科学会(島根県)	2014年9月4日 - 5日	国内
アポE欠損マウスに対する分散ヘスペレチンの抗動脈硬化作用	東口文治 菅澤典子 中山泰介 木下　肇 宅見央子 粟飯原賢一 黒部裕嗣 北川哲也	公益社団法人日本食品科学工学会　第61回大会(福岡県)	2014年8月28日 - 30日	国内
Stanford B型大動脈解離に対するステントグラフト治療の有効性	藤本鋭貴 筑後文雄 木下　肇 黒部裕嗣 神原　保 割石精一郎 加納正志 北市　隆 北川哲也	第104回日本循環器学会　中国・四国合同地方会(岡山県)	2014年7月18日 - 19日	国内
小児心臓手術時における経食道心エコーの合併症の検討	北市　隆 菅野幹雄 木下　肇 中山泰介 黒部裕嗣 神原　保 藤本鋭貴 川人伸次 坂田美穂 早渕康信 北川哲也	第50回日本小児循環器学会総会・学術集会(岡山県)	2014年7月3日 - 5日	国内
当院における過去13年間の小児心疾患患者死亡例の検討	清水信隆 真船　亮 林　泰佑 進藤考洋 平田陽一郎 犬塚　亮	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内

Extracardiac conduit TCPC(EC-TCPC)術後に心外導管による心房圧迫を評価し得た3例	真船　亮 犬塚　亮 林　泰佑 進藤考洋 平田陽一郎 清水信隆	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内
小児先天性心疾患術後に合併した乳糜胸の治療抵抗性の危険因子の検討	片山菜穂子 平田陽一郎 真船　亮 進藤考洋 林　泰佑 犬塚　亮 清水信隆 岡　明 平田康隆 小野　稔	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内
大動脈離断複合(type B)において3pの部分モノソミーを認めた1例	野木森宜嗣 平田陽一郎 林　泰佑 進藤考洋 犬塚　亮 清水信隆 関　正史 小川誠司 生井良幸 滝田順子 岡　明	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内
小児心筋症の予後不良因子の検討	進藤考洋 真船　亮 林　泰佑 平田陽一郎 犬塚　亮 清水信隆 波多野将 絹川弘一郎 木下　修 平田康隆 小野　稔	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内
Vector Flow Mappingを用いた左室内血流のエネルギー損失の計測　小児の基準値と年齢・心拍数・前負荷の影響	林　泰佑 板谷慶一 犬塚　亮 進藤考洋 平田陽一郎 清水信隆 宮地　鑑	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内
Berlin Heart Excor使用の小児重症心不全児に対するbridge to transplant治療	益澤明広 尾崎晋一 高岡哲弘 平田康隆 小野　稔 進藤考洋 林　泰佑 平田陽一郎 清水信隆 犬塚　亮 岡　明	第50回日本小児循環器学会総会・学術集会（岡山県）	2014年7月3日 - 5日	国内

乳児期のcriticalな僧帽弁膜症に対する外科治療	北市　隆 菅野幹雄 木下　肇 中山泰介 黒部裕嗣 神原　保 藤本鋭貴 北川哲也	第57回関西胸部外科学会学術集会(大阪府)	2014年6月19日 -20日	国内
感染性腹部大動脈瘤に対して上腸間膜動脈にSnorkeling EVARを施行した1例	木下　肇 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 北市　隆 北川哲也	第2回四国心臓血管外科フォーラム(愛媛県)	2014年5月31日	国内
二尖性大動脈弁に対する大動脈弁置換術時の上行大動脈壁の問題点	木下　肇 中山泰介 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 北市　隆 北川哲也	第42回日本血管外科学会学術総会(青森県)	2014年5月21日 -23日	国内
傍腎動脈腹部大動脈瘤に対するEVARの検討	藤本鋭貴 筑後文雄 中山泰介 木下　肇 菅野幹雄 黒部裕嗣 神原　保 割石精一郎 加納正志 北市　隆 北川哲也	第42回日本血管外科学会学術総会(青森県)	2014年5月21日 -23日	国内
エゼチミブ投与が動脈リモデリング・機能へ与える影響の検討	黒部裕嗣 菅澤典子 平田陽一郎 島袋充生 中山泰介 吉田恭史 松岡祐貴 木下　肇 佐田政隆 北川哲也	第42回日本血管外科学会学術総会(青森県)	2014年5月21日 -23日	国内
感染性腹部大動脈瘤に対して上腸間膜動脈にsnorkeling EVARを施行した1例	木下　肇 中山泰介 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 北市　隆 北川哲也	第42回日本血管外科学会学術総会（青森県）	2014年5月21日 -23日	国内

PPAR-γ Agonist Administration Attenuates inflammation In Patients With Aortic Aneurysm	H. KUROBE T. Motoki Y. Hirata M. Sugano T. Nakayama H. Kinoshita T. Kanbara E. Fujimoto T. Kitaichi T. Hori H. Sogabe M. Sata T. Kitagawa.	AATS Aortic Symposium 2014 (New York, NY, USA)	April 24 - 25, 2014	国外
The efficacy of endovascular treatment for type B aortic dissection	Hajime kinoshita Taisuke Nakayama Mikio Sugano Hirotsugu Kurobe Tamotsu Kanbara Eiki Fujimoto Takashi Kitaichi Tetsuya Kitagawa	AATS Aortic Symposium 2014 (New York, NY, USA)	April 24 - 25, 2014	国外
特異な臨床経過を認めた静脈血栓塞栓症の2 例	北市　隆 木下　肇 中山泰介 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 北川哲也	第34回日本静脈学会総会（沖縄県）	2014年4月17日 - 18日	国内
うっ滞性皮膚潰瘍を合併した下肢静脈瘤に対して血管内レーザー焼灼術の有効性	木下　肇 中山泰介 菅野幹雄 黒部裕嗣 神原　保 藤本鋭貴 北市　隆 北川哲也	第34回日本静脈学会総会（沖縄県）	2014年4月17日 - 18日	国内
初回IVIG不応川崎病症例における予後予測因子の検討	中釜　悠 犬塚　亮 柳澤敦広 稲冨　淳 林　泰佑 進藤考洋 平田陽一郎 清水信隆 張田　豊 岡　明	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内

小児に対する体外式心室補助循環装置の使用例の報告	進藤考洋 真船　亮 林　泰祐 平田陽一郎 犬塚　亮 清水信隆 平田康隆 村上　新 小野　稔	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内
過去10年間当院にて心内修復術を施行したASD/VSD有するDown症候群34例の後方視的検討	眞下秀明 平田陽一郎 林　泰佑 進藤考洋 犬塚　亮 清水信隆 岡　明 平田康隆 村上　新 小野　稔	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内
当院における過去10年間の左心低形成症候群症例の中期成績	平田陽一郎 真船　亮 林　泰祐 進藤考洋 犬塚　亮 清水信隆 岡　明 平田康隆 村上　新	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内
フォンタン循環患者における肝線維化と血清ヒアルロン酸値　門脈血流パターンとの関連	林　泰佑 進藤考洋 平田陽一郎 犬塚　亮 清水信隆 岡　明	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内
過去10年間当院にて心内修復術を施行したファロー四徴症患者の後方視的検討	野木森宜嗣 平田陽一郎 真船　亮 林　泰祐 進藤考洋 犬塚　亮 清水信隆 岡　明 平田康隆 村上　新	第117回日本小児科学会学術集会（愛知県）	2014年4月11日 - 13日	国内
胸部大動脈破裂に対する緊急TEVAR の検討	藤本鋭貴 筑後文雄 中山泰介 木下　肇 菅野幹雄 黒部裕嗣 神原　保 割石精一郎 加納正志 北市　隆 北川哲也	第114回日本外科学会定期学術集会（京都府）	2014年4月3日 - 5日	国内

Vascular remodeling effects by administrating Ezetimibe after arterial wire-injury in mice	Hirotsugu Kurobe Noriko Sugasawa Yoichiro Hirata Mitsuo Shimabukuro Taisuke Nakayama Takeshi Yoshida Mark W. Maxfield Tetsuya Kitagawa	ASCVTS(ISTANBUL, TURKEY)	April3 - 6, 2014	国内

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所 （学会誌・雑誌等名）	発表した時期	国内・外の別
Comparison of a Closed System to a Standard Open Technique for Preparing Tissue-Engineered Vascular Grafts.	Kurobe H Maxfield MW Naito Y Cleary M Stacy MR Solomon D Rocco KA Tara S Lee AY Sinusas AJ Snyder EL Shinoka T Breuer CK	Tissue Eng Part C Methods	2015 Jan	国外
Dissipative energy loss within the left ventricle detected by vector flow mapping in children: Normal values and effects of age and heart rate.	Hayashi T Itatani K Inuzuka R Shimizu N Shindo T Hirata Y Miyaji K	J Cardiol	2015, in press	国内
Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice.	Hara T Fukuda D Tanaka K Higashikuni Y Hirata Y Nishimoto S Yagi S Yamada H Soeki T Wakatsuki T Shimabukuro M Sata M	Atherosclerosis	2015, in press	国外

Total anomalous pulmonary venous connection with ccTGA and VSD: Can pulmonary artery banding avert pulmonary venous obstruction?	Hayashi T Hirata Y Inuzuka R Hirata Y	Pediatr Int	2015, in press	国外
Targeted imaging of matrix metalloproteinase activity in the evaluation of remodeling tissue-engineered vascular grafts implanted in a growing lamb model	Stacy MR Naito Y Maxfield MW Kurobe H Tara S Chan C Rocco KA Shinoka T Sinusas AJ Breuer CK	J Thorac Cardiovasc Surg	2014 Nov	国外
Comparison of the biological equivalence of two methods for isolating bone marrow mononuclear cells for fabricating tissue-engineered vascular grafts	Kurobe H Tara S Maxfield MW Rocco KA Bagi P Yi T Udelsman B Dean EW Khosravi R Powell HM Shinoka T Breuer CK	Tissue Eng Part C Methods	2014 Nov 14	国外
Well-organized neointima of large-pore poly(l-lactic acid) vascular graft coated with poly(l-lactic-co-ε-caprolactone) prevents calcific deposition compared to small-pore electrospun poly(l-lactic acid) graft in a mouse aortic implantation model	Kurobe H Tara S Rocco KA Maxfield MW Best CA Yi T Naito Y Breuer CK Shinoka T	Atherosclerosis	2014 Oct 17	国外
Evaluation of remodeling process in small-diameter cell-free tissue-engineered arterial graft	Tara S Kurobe H Maxfield MW Rocco KA Yi T Naito Y Breuer CK Shinoka T	J Vasc Surg	2014 Apr 15	国外

Increased Number of Hassall's Corpuscles in Myasthenia Gravis Patients with Thymic Hyperplasia	Naoko Matsui Izumi Ohigashi Keijirou Tanaka Mie Sakata Takahiro Furukawa Yasushi Nakagawa Kazuya Kondo Tetsuya Kitagawa	J Neuroimmunol	2014 Apr 15	国外
Iliac access conduit facilitates endovascular aortic aneurysm repair and ipsilateral iliofemoral bypass.	Kinoshita H Fujimoto E Sogabe H Fujita H Nakayama T Sugano M Kurobe H Kanbara T Kitaichi T Kitagawa T	J Med Invest	2014	国内
Serum hyaluronic acid concentration in Fontan circulation: correlation with hepatic function and portal vein hemodynamics.	Hayashi T Inuzuka R Shindo T Hirata Y Shimizu N Oka A	Pediatr Cardiol	2014	国外
Massive tricuspid regurgitation due to pacemaker-lead puncture of the tricuspid valve: successful diagnosis by 3-dimensional echocardiography	Rina Tamai Tomoya Hara Hirotsugu Yamada Susumu Nishio Mika Bando Junko Hotchi Shuji Hayashi Toshiyuki Niki Tetsuya Kitagawa Masataka Sata	J Med Ultrasonics	2014	国内
Norwood手術における Blalock-Taussigシャント－実験から臨床への展開－	北市　隆 菅野幹雄 木下　肇 中山泰介 黒部裕嗣 神原　保 藤本鋭貴 北川哲也	胸部外科	2014	国内
画像診断　Q&A	北川哲也 曽我部仁史	胸部外科	2014	国内

（注１）発表者氏名は、連名による発表の場合には、筆頭者を先頭にして全員を記載すること。

（注２）本様式はexcel形式にて作成し、甲が求める場合は別途電子データを納入すること。

Ⅳ．学会等発表実績の刊行物・別刷

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Comparison of a Closed System to a Standard Open Technique for Preparing Tissue-Engineered Vascular Grafts

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We developed a prototype for a closed apparatus for assembling tissue-engineered vascular grafts (TEVGs) with the goal of creating a simple operator-independent method for making TEVGs to optimize safety and enable widespread application of this technology. The TEVG is made by seeding autologous bone marrow-derived mononuclear cells onto a biodegradable tubular scaffold and is the first man-made vascular graft to be successfully used in humans. A critical barrier, which has prevented the widespread clinical adoption of the TEVG, is that cell isolation, scaffold seeding, and incubation are performed using an open method. To reduce the risk of contamination, the TEVG is assembled in a clean room. Clean rooms are expensive to build, complex to operate, and are not available in most hospitals. In this investigation, we used an ovine model to compare the safety and efficacy of TEVGs created using either a standard density centrifugation-based open method or the new filter-based closed system. We demonstrated no graft-related complications and maintenance of growth capacity in TEVGs created using the closed apparatus. In addition, the use of the closed system reduced the amount of time needed to assemble the TEVG by ~50%. Adaptation of similar methodologies may facilitate the safe translation and the widespread use of other tissue engineering technologies.

Introduction

We demonstrated the feasibility of using tissue-engineered vascular grafts (TEVGs) in the surgical repair of congenital cardiac anomalies in children undergoing open heart surgery[1,2] and continue to target the pediatric patient population to take advantage of its growth potential.[3–5] To construct the TEVG, autologous bone marrow is harvested AU3 ▶ and the mononuclear cells (BM-MNCs) are isolated and see (glycolic acid) fibers knitted into a tube and coated with a 50:50 copolymer of ε-caprolactone and L-lactic acid.[1,2] The seeded construct is then incubated for 2 h before implantation as a vascular conduit.[1,2] Cell harvest, isolation, seeding, and incubation are performed during a single surgical procedure followed by surgical implantation of the TEVG. Thus, there is a significant benefit associated with minimizing the amount of time needed to assemble the TEVG.

The clinical utility of the TEVG is limited by the need to use a clean room, specifically an International Organization for Standardization (ISO) class 7 laboratory, to assemble the TEVG, which involves using an open method for cell isolation, scaffold seeding, and incubation.[6] The use of an open technique increases the risk of contamination and introduces operator variability to the process of making the TEVG. Clean rooms are expensive to build and maintain and require significant manpower to operate in compliance with good manufacturing process standards. We have previously demonstrated that a simpler, alternative filtration-based method can be used to isolate BM-MNCs instead of the standard density centrifugation method that we currently use to isolate these cells.[7] The filter-based cell isolation method has the added advantage of being performed more rapidly and can serve as the basis for a closed system processing.[7] The use of a closed system would minimize the risk of contamination and improve the safety profile for the TEVG. We demonstrated the feasibility of using this alternative filter-based cell isolation method to successfully create neovessels using a murine model.[7] Herein, we discuss the results of an investigation designed to compare the safety and efficacy of TEVGs assembled using the standard open method versus TEVGs assembled using the closed apparatus. Our goal is to create a simple, safe,

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operator-independent apparatus for assembling TEVGs that would eliminate the need for the use of a clean room.

Materials and Methods

Scaffolds

Scaffolds measuring 13 cm length and 12 mm inner diameter with wall thickness measuring ~700 μm were fabricated from poly(glycolic acid) fibers, which were knitted into a tube, and coated with a 50:50 copolymer of ε-caprolactone and L-lactic acid (Gunze Limited).[1,2] Scaffolds had 80% porosity with pore sizes from 100 to 200 μm. The scaffolds were packaged and gas sterilized using ethylene oxide before being shipped from the manufacturer for use in the study.

Bone marrow harvest

Twelve juvenile, female, Dover lambs (weight 20–30 kg) were used in this study (Morris Farms). All animals received humane care in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee at the Yale University approved the use of animals and all procedures described in this study. Under general anesthesia, 50 mL of bone marrow was harvested from each animal as previously described.[8] A sample of the bone marrow was obtained, and the cell viability was measured using trypan blue exclusion.

Cell isolation, scaffold seeding, and incubation (open method)

Under a biosafety hood, using sterile technique, BM-MNCs were isolated from 50 mL of heparinized bone marrow using density centrifugation in the Histopaque 1077 (Sigma-Aldrich).[8] BM-MNCs were then seeded onto the scaffold using the vacuum (−50 mm Hg) seeding method as previously described.[6] The seeded scaffold was then placed in a sterile container and bathed in autologous serum and incubated (37°C, 5% CO_2, 95% relative humidity, 760 Torr) for a minimum of 2 h before implantation.[1,2] A $1 \times 1\ cm^2$ section of the seeded incubated scaffold was excised, fixed, and embedded using glycol methacrylate.[9] The sections were stained with the Lee's methylene blue, and the number of attached cells was determined. The total time for cell isolation, scaffold seeding, and incubation was recorded.

Cell isolation, scaffold seeding, and incubation (closed method)

We developed a prototype for a closed disposable system designed to (1) isolate BM-MNCs from autologous bone marrow using a filtration method, (2) seed the scaffold with the BM-MNCs using a vacuum seeding technique, and (3) incubate the seeded scaffold before surgical implantation (Fig. 1). The system uses a nonwoven polyester fiber filter that works, in part, by interception to trap the BM-MNCs

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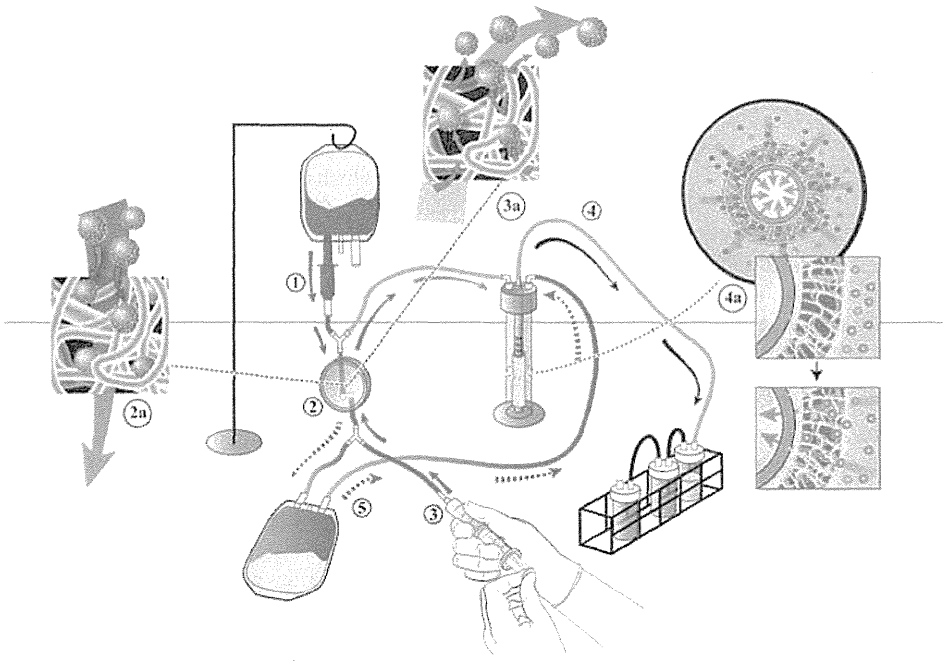


FIG. 1. Schematic representation of the closed disposable seeding system for assembling tissue-engineered vascular grafts (TEVGs). Heparinized bone marrow is added to the bone marrow bag. The bone marrow bag is then suspended at a head-height of 18 inches and the bone marrow is transferred into the drip chamber, which acts to filter out large particles (1). The bone marrow then flows out through the drip chamber filter and through the cell harvest filter (2), which entraps bone marrow-derived mononuclear cells (BM-MNC) (2a). The effluent is collected in the effluent bag. Sixty milliliters of harvest solution (10% Dextran 40) is then back-flushed through the filter (3), releasing entrapped BM-MNC (3a) and collecting them in the seeding chamber. The BM-MNCs are then seeded onto the scaffold using vacuum seeding (4, 4a). The effluent is then transferred to the seeding chamber thus bathing the seeded scaffold (5). At that time, the tubing is heat sealed and the TEVG is placed in the incubation chamber for 2 h, at which point it is ready for surgical implantation. Color images available online at www.liebertpub.com/tec

◀ AU3

Pall Corporation). The trapped cells are eluted off the filter using 60 cc of elution solution (10% Dextran 40; Pall Corporation). The cells were seeded onto the scaffold using vacuum seeding (−50 mm Hg) and incubated in an autologous effluent for a minimum of 2 h before implantation. A $1 \times 1\,cm^2$ section of the seeded incubated scaffold was excised, fixed, and embedded using glycol methacrylate.[8] The sections were stained with the Lee's methylene blue, and the number of attached cells was determined. The total time for cell isolation, scaffold seeding, and incubation was recorded.

Surgical implantation

The TEVGs were implanted as intrathoracic inferior vena cava (IVC) interposition grafts as previously described.[8] Each anastomosis was marked with a titanium ring to facilitate their identification during computed tomography (CT). The IVC interposition graft model is a high-flow low-pressure model that we have developed for evaluating vascular grafts designed for use in congenital heart surgery, where most vascular grafts are used in high-flow low-pressure circuits, such as the pulmonary or Fontan circulation.[8,10] Animals were divided into two groups (open method versus closed system) for the study, and a total of 12 autologous grafts ($n=6$/group) were implanted as end-to-end IVC interposition grafts as part of this study. All lamb surgeries were performed under general endotracheal anesthesia as previously described.[8] Animals were anticoagulated with heparin (100 U/kg) at implantation. No postoperative antiplatelet or anticoagulant agents were used postoperatively.

In vivo *CT angiography*

In vivo 64-slice x-ray CT angiography (Discovery NM-CT 570c; GE Healthcare) with iodinated contrast (350 mgI/mL, Omnipaque; GE Healthcare) was used to assess graft dilatation, narrowing, and luminal and longitudinal growth at 2 and 6 months following the TEVG implantation. Animals were mechanically ventilated with 35% oxygen, 65% nitrous oxide, and 1–3% isoflurane (Venturi; Cardiopulmonary Incorporated) while hemodynamics were continuously monitored (IntelliVue MP50; Philips). A 5F catheter was placed in a hind limb vein for the administration of fluids and CT contrast agent. Intravenous contrast injections were performed using a power injector (Stellant D; MEDRAD). Lambs were kept NPO on the night before CT imaging and were given an intravenous 20 cc/kg bolus of normal saline after induction of anesthesia to standardize their hydrational status. Images were acquired at a slice thickness of 0.625 mm, at 300 mA, and 120 kVp. TEVG luminal diameter, wall thickness, luminal volume, and length were quantified at 2 and 6 months using commercially available software (Advanced Workstation v4.4; GE Healthcare).

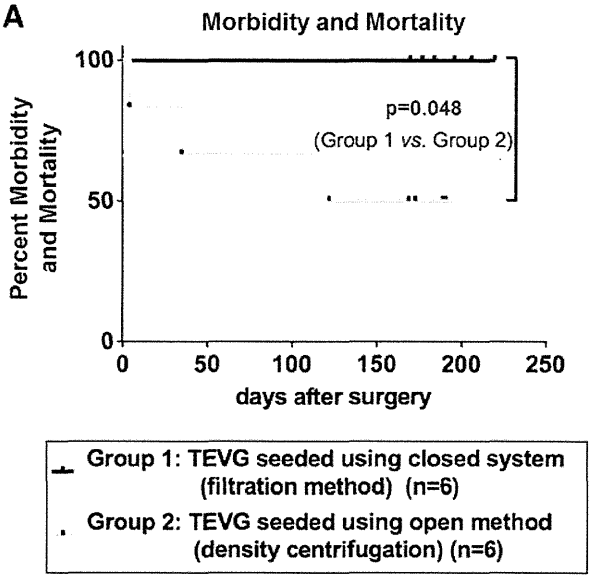
Histology

The animals were euthanized and the vascular grafts were pressure fixed with formalin and harvested after the 6-month implantation as previously described.[8] Tissue were embedded in paraffin, sectioned (5 μm sections), and stained with hematoxylin and eosin (H&E), Masson's trichrome, Elastica van Gieson, Hart's, Alcian blue, and Von Kossa.

Statistical analyses

Statistical analyses were performed with the Student's *t*-test for continuous variables with normal distribution and the chi-square test for dichotomous variables. One-way analysis of variance was used to determine significant differences between three or more groups. *p*-Values less than 0.05 indicated statistical significance. Numeric values are listed as mean ± SD.

A



B Graft related complications

Complications \ Groups	(Open Method) Density Centrifugation (n=6)	(Closed System) Filtration (n=6)
Acute thrombosis	0	0
Stenosis	2	0
Aneurysm	0	0
Infection	0	0

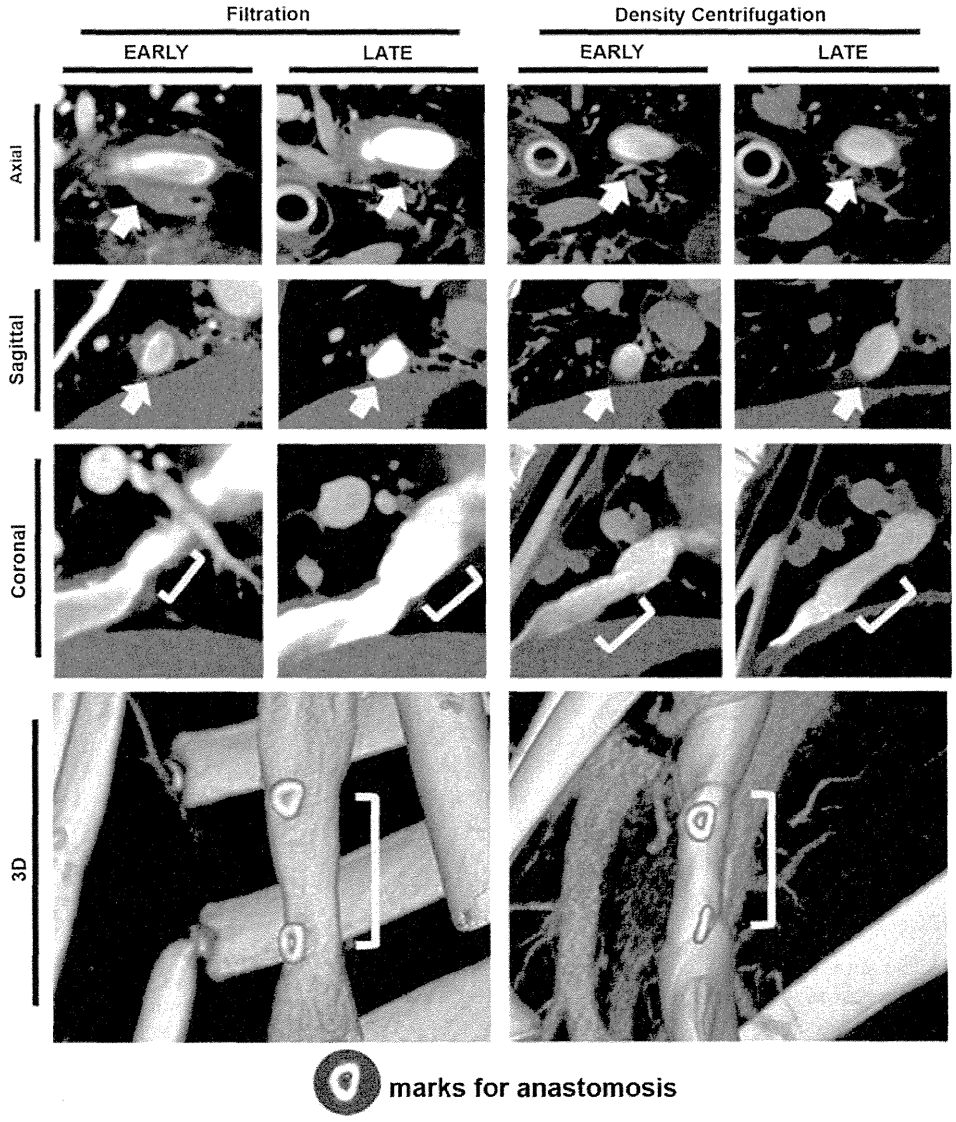
FIG. 2. Summary of morbidity and mortality data for TEVG implanted in the lamb model. TEVG were implanted as intrathoracic IVC interposition grafts and monitored over a 6-month time course. A total of 12 TEVG were implanted and divided into two equal groups ($n=6$/group). Group 1 consisted of TEVG seeded using a closed filter-based system and group 2 consisted of TEVG seeded using an open density centrifugation-based method. **(A)** Survival curves: There was one surgical complication, which occurred in the open method group. It consisted of a neurological injury that resulted from a prolonged clamp time. Due to the severity of the neurological deficit, the animal required euthanasia. **(B)** Graft-related complications: There were two graft-related complications (critical stenosis), which developed in the open method group and also required euthanasia. No deaths or graft-related complications occurred in the TEVG assembled using the closed system. IVC, inferior vena cava.

Results

We used either an open (density centrifugation) or a closed (filter-based) system to isolate BM-MNCs, seed the scaffold, and incubate the seeded construct before TEVG implantation as an intrathoracic IVC interposition graft in a juvenile in lamb model. Cells were seeded using a vacuum-seeding (−50 mm Hg) technique. Results of the trypan blue exclusion of the bone marrow cells demonstrated cell viability of >90% for both the groups. Results of the cell attachment studies demonstrated that a similar number of cells were seeded onto scaffolds in both the groups as measured by the Lee's methylene blue staining on glycol methacrylate-fixed tissue (density centrifugation: 2900±2050 cells/mm^2; filtration: 2630±1590 cells/mm^2, $p=0.77$). Total procedure time was significantly decreased using the closed system (average 2 h, 17 min) compared with the open method (average 4 h, 28 min). The time saving was primarily the result of using the filter-based method instead of the density centrifugation method for isolating BM-MNCs.

There was one surgical complication resulting from a prolonged clamp time that caused a neurological injury, which necessitated early sacrifice of one of the animals from the open method group. Two additional animals in the open method group developed critical stenoses, which caused portal hypertension, hepatic dysfunction, and ascites requiring early sacrifice. All six lambs implanted with TEVG assembled using the closed disposable seeding apparatus survived surgery and demonstrated no evidence of any graft-related complications throughout the 6-month time course of the study (Fig. 2). ◀F2

CT angiography was performed at early (2 months) ($n=11$) and late (6 months) ($n=9$) time points. All TEVGs that underwent CT angiography at the two separate time points ($n=9$) demonstrated both luminal growth and increase in graft length without radiological evidence of thrombosis or aneurismal dilation. The mean graft diameter increased 19% from an average diameter of 10.7±2.3 to 12.7±1.6 mm. The graft length increased 16% from a mean length of 20.9±1.3 to 24.2±2.3 mm. The graft volume increase of 48%, from



AU4▶ **FIG. 3.** Computer tomography (CT) analysis of TEVG implanted in lamb model. Serial CT imaging was used to assess the change in size and shape of the TEVG implanted in juvenile lambs over a 6-month time course. All surviving animals were serially imaged at early (2 months) and late (6 months) time points. At the early time point, the TEVG showed thickening of the graft wall accompanied in some instances by graft luminal narrowing ($n=11$). There was no radiographic evidence of thrombosis in any graft. The graft luminal volume increased over the course of the experiment in all ($n=9$) animals that survived the 6-month time course. There was no radiographic evidence of aneurismal dilation in any grafts. Color images available online at www.liebertpub.com/tec AU6▶

2128 ± 927 to $3145 \pm 739\,mm^3$, was similar to the 36% increase in volume of the native superior vena cava over the same time period. The graft wall thickness decreased from a mean of 4.4 ± 1.0 to 2.7 ± 1.0 mm, which approached the F3 ▶ thickness of the native IVC (Fig. 3).

Qualitative histological assessment performed on tissue explanted 6 months after implantation demonstrated a TEVG wall that resembled native tissue given the presence of three distinct layers, including a neointima, neomedia, and neoadventitia. H&E staining revealed cellular architecture in the grafts resembling that of a native vessel. Masson's trichrome showed robust collagen formation in both the groups. There was no evidence of ectopic calcification in any specimens (Von Kossa). Elastin staining was present in both the groups but not as well developed or organized as in the native IVC (Elastica Van Gieson and Hart's). Glycosaminoglycan staining was seen in both the groups F4 ▶ (Alcian blue) (Fig. 4).

Discussion

In this study, we evaluated a prototype for a closed disposable seeding system for constructing tissue-engineered vascular grafts and compared it with our currently used open system. We performed a study comparing the incidence of graft-related complications and growth capacity of TEVG constructed using either the closed filter-based seeding system or the open density centrifugation-based methodology. Using an ovine model, we demonstrated no graft-related complications in the TEVG constructed using the closed disposable seeding system and confirmed the growth capacity of the TEVG using serial CT angiography. Taken together, these data support the feasibility of creating a closed system for assembling TEVGs and suggest that such a system could improve both the safety and the clinical utility of this technology by creating a method that is simpler, faster, and operator-independent.

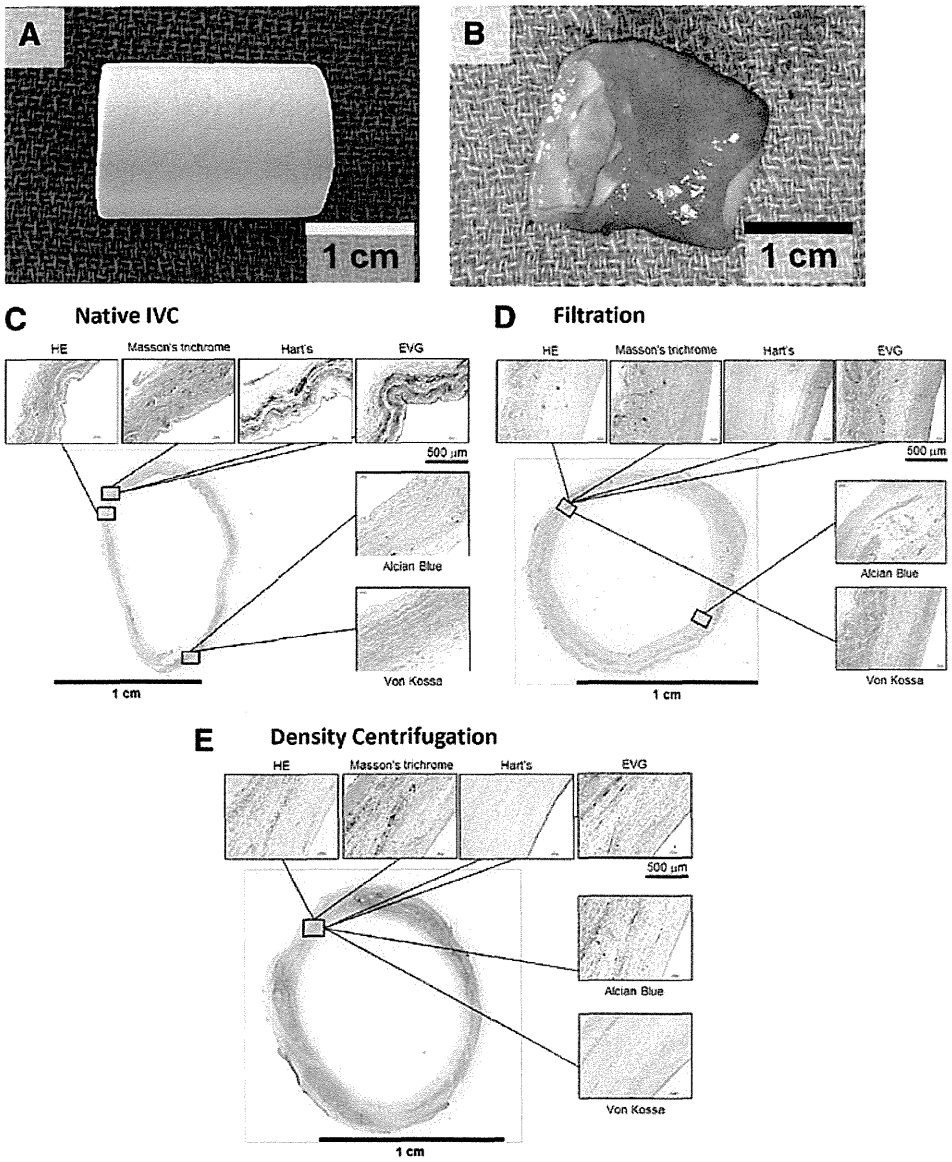


FIG. 4. Histological analysis of TEVG: TEVGs in lambs explanted at 6 months after implantation resemble native blood vessels. **(A)** Scaffold. Before surgical implantation, all grafts were measured and cut to 20 mm in length. **(B)** At time of explantation, seeded scaffolds had transformed into neovessels resembling native inferior vena cava. **(C–E)** H&E staining revealed cellular architecture in the grafts resembling that of native vessels. Masson's trichrome staining showed robust collagen formation in both the groups. There was no evidence of ectopic calcification (Von Kossa). Elastin staining was present in both the groups but not as well developed as seen in the native IVC (Elastica Van Gieson [EVG] and Hart's stain). Glycosaminoglycan staining was seen in both the groups (Alcian blue stain). H&E, hematoxylin and eosin. Color images available online at www.liebertpub.com/tec

Upon initiation of our clinical trial in the United States, we switched from a manual seeding method to a vacuum seeding method in an attempt to reduce the operator variability and mitigate the risks associated with making a TEVG. We performed a series of experiments demonstrating the equivalence of both techniques before introducing this technology to the clinic.[6] This sort of incremental process improvement is critical to rational design of a better safer product. In this investigation, we evaluate the next step in process improvement for TEVG production. We developed a closed disposable system based on filtration method for isolating BM-MNCs. The resulting closed disposable system is simpler, faster, and operator-independent. In our previous work, preliminary characterization of this methodology demonstrated that it was feasible. However, we noted differences in the subpopulations of the BM-MNCs, including increased numbers of red blood cells in the BM-MNCs isolated using the filter-based method.[7] Results of our current study demonstrate that despite these differences in the cell populations we were able to create viable neovessels using either technique. Furthermore, the absence of any graft-related complications and the preservation of the growth potential in the group of TEVGs created using the closed disposable seeding system highlight the safety and efficacy of this methodology.

The TEVG described herein is the first man-made vascular graft with growth potential. Results of our pilot study evaluating the use of the TEVG in congenital heart surgery confirmed the growth capacity of the TEVG in humans.[1,2] This has significant implications for children, particularly those with congenital heart disease.[3–5] Before the potential of the TEVG can be fully realized, simpler, safer, and more rapid methods for assembling TEVGs (i.e., cell isolation, cell seeding, and incubation) need to be developed. Development of a closed disposable seeding system would overcome a critical barrier and would make this technology available to many more patients. Adoption of a similar approach for creating other tissue-engineered products could serve as a paradigm for process improvement, which would improve both the safety and the clinical utility of other tissue-engineered products, thereby facilitating the translation of these technologies from the bench to the clinic.

Acknowledgments

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Disclosure Statement

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AU1: Please review all authors' surnames for accurate indexing citations.
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AU3: Please fix the expansion of the acronym BM-MNCs: "bone marrow is harvested and the mononuclear cells" or "bone marrow-derived mononuclear cells"?
AU4: Please fix the expansion of the acronym CT: "computed tomography" or "computer tomography"?
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AU6: Please mention what the "arrows" and "]" indicate in Fig. 3.