ORIGINAL ARTICLE

Braz J Cardiovasc Surg 2007; 22(1): 81-86

Hemodinamic behavior of arterial anastomosis using fibrin sealant. Experimental study in swine

Avaliação hemodinâmica de anastomoses arteriais reforçadas com selante de fibrina. Estudo experimental em suínos

Eduardo Augusto Victor ROCHA[1], Cláudio de SOUZA[2]

RBCCV 44205-872

Abstract

Objectives: **To evaluate the flow, tear pressure, the need of reinforcement stitches in sutured arteries reinforced or not using fibrin sealant after a cross-section.**

Method: **Tissucol® fibrin sealant was used. The femoral and carotid arteries of seventeen swine from the same breed (weighing from 15 to 20 kg) were cross-sectioned after heparinization and subjected to anastomoses using a single continuous plane of prolene 7-0. We worked with 68 artery samples, 34 in the Treatment Group and 34 in the Control Group. For each animal, one carotid and one femoral artery randomly received fibrin sealant with the contralateral side being used as a control. The need and the number of reinforcement stitches were recorded. Ten minutes after protamine infusion, the animals were sacrificed and the arteries were catheterized. The arteries were measured and placed on a flow meter. The arteries were then subjected to air infusion at increasingly higher pressures (stepwise increases of 25 mmHg), the grafts were dipped in saline solution, the first air leakage was observed and the tear pressure recorded.**

Results: **The external diameters and thickness of the arteries were similar in both the Treatment and Control Group. There was no significant difference between the groups regarding the tear pressure (p=0.329), flow rate (p=0.943) and the number of samples with a tear pressure above 200 mmHg. However, the sealant reduced the number of reinforcement stitches necessary (p=0.029).**

Conclusion: **Fibrin sealant reduces the need of additional stitches.**

Descritors: **Fibrin tissue adhesive. Anastomosis, surgical, methods. Hemostasis.**

1. Master in surgery. Cardiovascular surgeon
2. PhD in surgery. Adjunct Professor of the Medicine School of UFMG.

Work carried out in the Medicine School of the Federal University of Minas Gerais, MG.
Correspondence address:
Eduardo Augusto Victor Rocha. Rua Paracatu, 838 sala 209 - Santo Agostinho - Belo Horizonte - MG. CEP: 30180-090.
E-mail: e.rocha@gcsnet.com.br

Article received in October 23rd, 2006
Article accepted in February 15th, 2007

Resumo

Objetivo: **Avaliar o fluxo, a pressão de ruptura, a necessidade de pontos de reforço, em artérias suturadas, reforçadas ou não com selante de fibrina após uma secção transversal.**

Método: **Utilizou-se como selante o Tissucol®. Dezessete suínos, Landrace ligth, pesando entre 15 e 20 kg, tiveram suas artérias femorais e carótidas seccionadas após heparinização, anastomosadas em plano único contínuo de prolene 7-0. Usamos 68 amostras arteriais, 34 no grupo tratamento e 34 no grupo controle. Uma artéria carótida e uma femoral receberam, selante de fibrina, aleatoriamente; o lado contralateral foi o controle. Anotava-se a necessidade e o número de pontos de reforço. Após 10 minutos da infusão de protamina, sacrificavam-se os animais, cateterizavam-se as artérias. Estas artérias foram mensuradas, colocadas num fluxômetro, onde se avaliava a velocidade do fluxo. As artérias foram submetidas à infusão de ar, com pressões sucessivamente mais elevadas, mergulhadas em solução de NaCl a 0,9%, observando-se o primeiro vazamento aéreo. Analisaram-se os dados estatisticamente.**

Resultados: **Os diâmetros externos e a espessura das artérias, além da pressão de ruptura e número de amostra com ruptura superior a 200mmHg, foram semelhantes. Contudo, o selante diminuiu o número de pontos de reforço.**

Conclusão: **O selante de fibrina reduz a necessidade de pontos adicionais.**

Descritores: **Adesivo tecidual de fibrina. Anastomose cirúrgica, métodos. Hemostasia.**

INTRODUCTION

Even with the frequent combination of sealants with arterial sutures, the number of experimental works in the literature on this subject is small. The first reported use of sealants in cardiovascular surgery was by Brawnwald in 1966 [1,2]. Sealants are routinely utilized as: hemostatic agents [3-7], on arterial sutures [8,9], reinforcement in the surgical treatment of aorta diseases [10-12] and with other cardiac structural diseases, such as interventricular communications (IVC) and after acute myocardial infarction and congenital heart disease. Several experimental studies have been made to perform arterial anastomoses without sutures [13,14]. Other studies questioned its angiogenic effects [15].

However, no research has evaluated the reinforcement that sealants provide to arterial sutures, even though many surgeons use sealant for this purpose. Fibrin sealant, as it is derived from blood, presents risks inherent to its use, such as infections similar to those caused by blood transfusions, including parvovirus B19 [16], factor V and antithrombin antibody deficiency; as well as the risk of embolization. Thus, it is necessity to verify the efficacy of fibrin sealant use. This study has the objective of evaluating the rupture pressure, the flow, and also the necessity of reinforcement stitches in arteries sutured after sectioning, reinforced using Tissucol® fibrin sealant or not.

METHODS

All animals were treated following the ethics norms of the Brazilian College of Animal Experimentation (COBEA) with the study design being approved by the Surgery Department of the Medical School of UFMG. Seventeen Landrace light swine with weights ranging from 15 and 20 kg, had their femoral and carotid arteries occluded with surgical clamps and sectioned transversely. Anesthesia was achieved by an infusion of Ketamine (15 mg/kg intramuscular) and Xylazine (12.5 mg/kg intramuscular). Catheterization of one peripheral vein was attained using a Jelco® nº 20 catheter and Pentobarbital was endovenously infused (12.5 mg/kg) with heparinization being achieved using 1mg per kg of weight.

Subsequently, the arteries were anastomosed utilizing continuous polypropylene 7-0 sutures on a single plane (Figure 1).

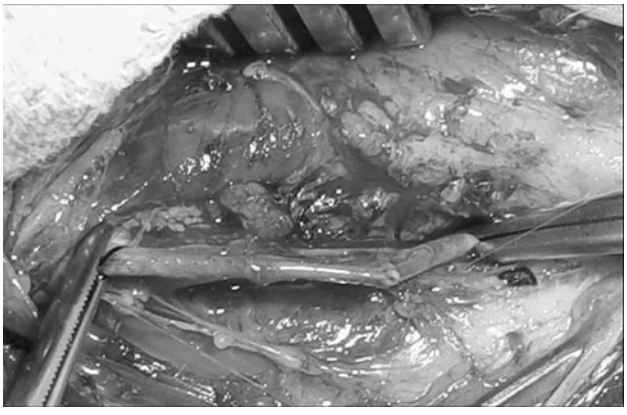

Fig. 1 – Final appearance of the arterial anastomosis

Sixty-eight arterial samples were studied, divided in two groups: 34 in the Treatment Group and 34 in the Control Group. For each animals, one carotid artery and one femoral artery were randomly chosen to receive 1 mL of fibrin sealant along the anastomosis, with the contralateral side being the control (Figure 2).

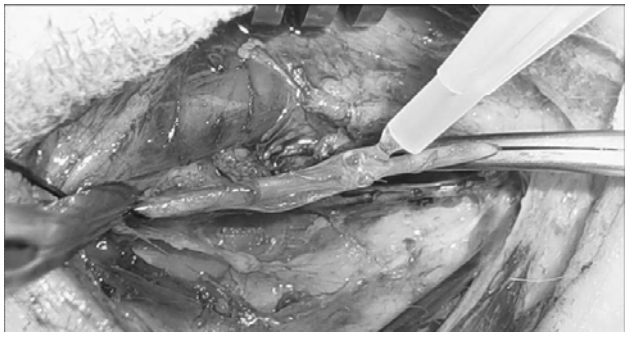

Fig. 2 – Fibrin sealant being applied to an anastomosis

After declamping, the necessity and number of additional sutures required to achieve adequate hemostasis of the anastomosis were recorded. Bleeding with jets of blood was reinforced.

Protamine was infused for 10 minutes after which the animals were submitted to euthanasia using deep anesthesia and an infusion of KCl until ventricular fibrillation. Subsequently, segments of arteries comprising of 1 cm either side of the anastomoses were dried. These arteries had their diameter and thickness measured using precision calipers when submitted to a pressure of 25 mmHg. The artery samples were catheterized and rinsed with 10 mL of 0.9% NaCl and placed in the flowmeter, where the flow speed was evaluated. The time for 10 mL of 0.9% NaCl saline solution to flow through the segment from a height of 50cm was recorded with the result given in seconds (Figure 3). Immediately after, the arteries were immersed in 0.9% NaCl solution and submitted to an air current at pressures increased by intervals of 25 mmHg (Figures 4 and 5).

At the exact moment at which an air leak was observed, the rupture pressure was recorded. The numbers of samples with rupture pressures of greater than 200 mmHg were compared. The pressure of 200 mmHg was chosen as the cut-off point after considering the systemic pressure.

However, only when the pressure increased to 300 mmHg was an absence of rupture recorded. The sample size was demonstrated to be statistically adequate. For statistical analysis, the Epi Info data management system was utilized. All the results were submitted to statistical hypothesis tests considering each parameter studied. Continuous variables were evaluated by the Student t-test or equivalent non-parametric Kruskal-Wallis test (when indicated), and categorical variables by the Chi-square test (with Yates's correction) or Fisher's exact test (when indicated), as well as the *t* test. For all analyses, a level of significance of 5% ($\alpha = 0.05$) was considered significant.

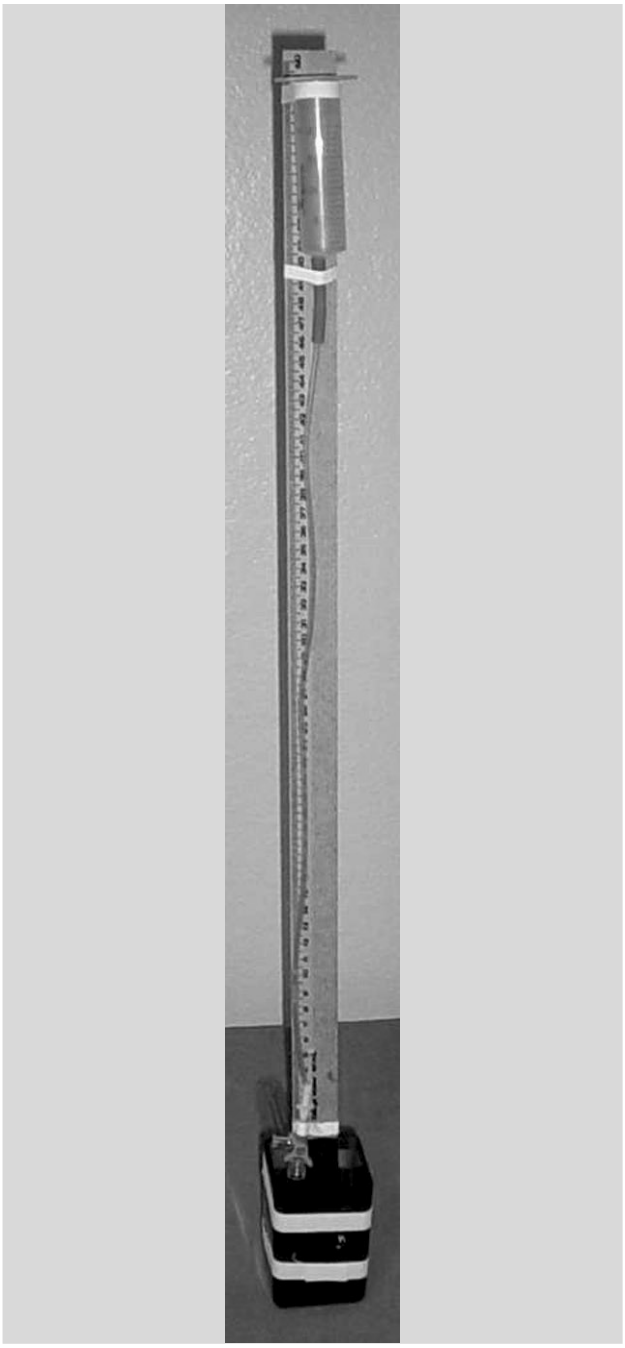

Fig. 3 – Mounted flowmeter

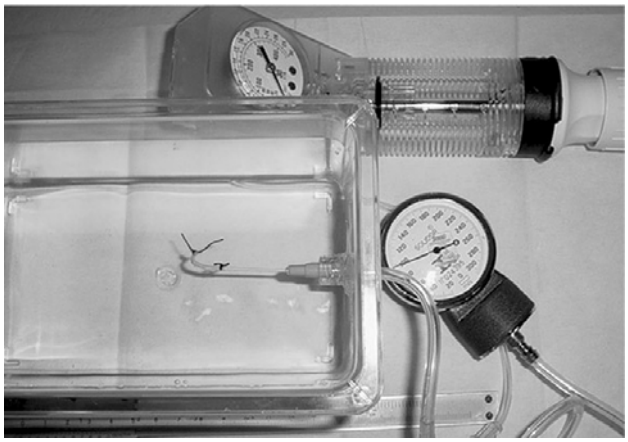

Fig. 4 – Arterial sample showing the rupture pressure

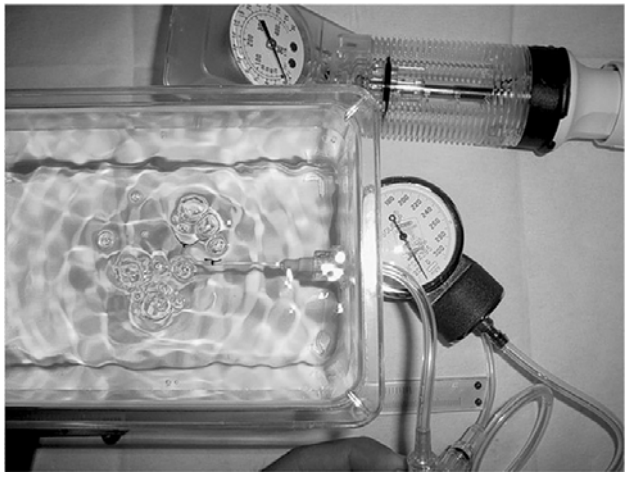

Fig. 5 - Arterial sample showing the moment of rupture and air leak

statistically significative (p=0.943). The mean external diameters were similar in both groups, with 2.185 mm in the Control Group and 2.276 mm in the Sealant Group (p=0.0557; Student t-test). The mean thicknesses of the arterial grafts were similar too; 0.029 mm in the Control Group and 0.030 mm in the Sealant Group (p=0.544; Student t-test). The mean time to prepare anastomoses was similar: 303.23 seconds for the Sealant Group and 293.85 seconds for the Control Group (p=0.59; Student t-test). The number of grafts with rupture pressures greater than 200 mmHg was 22 for both groups.

Table 1 illustrates the results obtained in this study.

Table 1. Data of the experience.

Data	Sealant	Control	p-value
Arteries with reinforcement sutures	08	18	0.025
Ruptured arteries	22	18	0.460
Mean rupture pressure (mmHg)	75	105	0.347
Mean flow time (seconds)	258.03	383.18	0.943
Mean external diameter (mm)	2.185	2.275	0.557
Mean arterial thickness (mm)	0.031	0.029	0.544
Mean time of anastomosis (seconds)	303.23	293	0.66
Arteries with rupture pressures ≥ 200 mmHg	22	22	0.8

RESULTS

The number of anastomoses requiring reinforcement sutures was 8 in the Sealant Group (n=34) and 18 in the Control Group (n=34) giving a significant difference demonstrated using chi-square with Yates's correction (p=0.025).

The mean arterial rupture pressure was 75.72 mmHg in the Sealant Group and 105.88 mmHg in the Control Group. The number of arteries that ruptured was 22 in the Sealant Group and 18 in the Control Group, without statistical significance calculated using chi-square with Yates's correction (p=0.460). The mean time spent for 10 mL of 0.9% NaCl to flow from a height of 50 cm was 383.18 seconds for the Control Group and 258.03 seconds for the Sealant Group. This difference, evaluated by Student t-test was not

DISCUSSION

Over the last ten years, several studies have suggested that sealants may reinforce saphenous graft walls or aorta walls in cases of dissection and for surgically corrected post-infarction IVC. In this study, insufficient data to support this hypothesis were found. The rupture pressure of grafts treated using the sealant was similar to untreated grafts (p=0.347). It was not possible to compare these data with published results as there are no publications on this theme. Thus, in this study the capacity of fibrin sealant to reinforce arterial anastomoses was not confirmed. The technique of rinsing arterial samples with 0.9% NaCl before checking the flow rate may have contributed to an absence

of significant differences of flow time between the groups even though saline solution has a lower viscously than blood. The objective of the study was simply to compare the arterial flow in vessels that used the sealant with the Control Group. Some authors utilizing an *ex vivo* model suggest that fibrin sealant can reinforce saphenous graft walls, making microscopic analysis of the saphenous graft submitted to a continuous flow at sub-systemic pressures (60 mmHg) [17]. This creates an artificial situation, as fewer microscopic lesions occur using sealants however, extrapolation of the results to clinical practice is difficult. In this work, no statistical difference between the flows of the two groups was evidenced, maybe, because each graft was irrigated with 10 mL of 0.9% NaCl before the flow test, as specified in the initial design protocol. The intention of using this method was to avoid any possible interference of blood clots, formed during resection of the samples, on the rupture pressure. This process made any conclusion about the embolic potential of fibrin sealant complicated, due to the possibility of reducing the arterial lumen; possible blood clots caused by the fibrin sealant passing through small orifices in the suture line. There are works that prove that the higher the concentration of thrombin, the quicker the fibrin is stabilized. The higher the concentration, the greater is the risk of emboli. Frost-Arner et al. [18] demonstrated that lower concentrations of thrombin increase the patency of anastomoses (Figure 6).

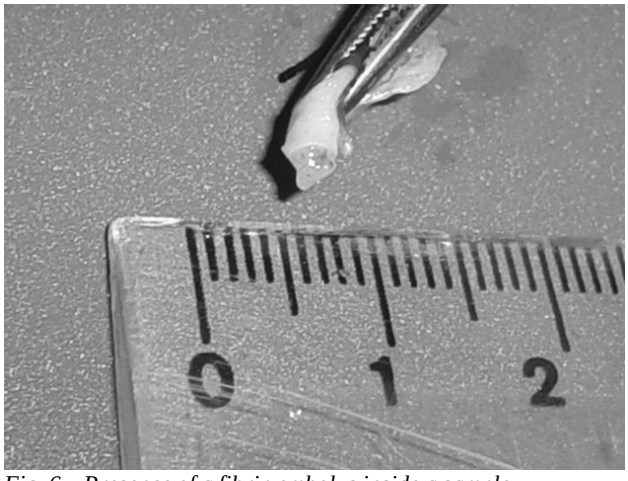

Fig. 6 – Presence of a fibrin embolus inside a sample

Although the indication of reinforcement sutures is based on the researcher's impression, the data are valid because all the experiment was performed by a single surgeon. The diminution of the number of reinforcement sutures in the suture line demonstrates the power of the sealant (p=0.029). This reduction of reinforcement sutures may have an impact on the result and on the cost, but this premise was not tested in this work. Reicher et al. [19] managed to reduce the number of sutures of arterial anastomoses using fibrin sealant. These authors demonstrated a reduction in the systolic velocity at Doppler and increase in the internal area of the anastomosis in a group treated with sealant. The authors, however, used different surgical techniques for the groups, creating more than one variable, making a definitive conclusion difficult.

Sealants have been widely utilized for their hemostatic effect [20-23]. Per-operative bleeding can decrease, as was suggest in the works of Taylor et al. [20], Unlu et al. [21] and Tawes et al. [22]. However, we can not confirm this statement with data from this current study. The blood loss was not measured as this was not included in the initial protocol and so the Control and Sealant Groups were not compared. The small quantity of blood loss, after declamping healthy arteries, submitted to technically simple suturing, uninspired us in respect to measuring this variable, however, we believe a study with this objective to be pertinent.

Autologous fibrin collection systems [23] may minimize the risk of infection, making the utilization of sealants safer, but these are preliminary studies, with material that is not commercially available yet.

CONCLUSION

Fibrin sealants reduced the necessity of additional sutures however they did not improve the rupture pressure or the arterial flow in the study group.

REFERENCES

1. Braunwald NS, Gay W, Tatooles CJ. Evaluation of crosslinked gelatin as a tissue adhesive and hemostatic agent: an experimental study. Surgery. 1966;59(6):1024-30.

2. Souza C. Uso de adesivos biológicos em anastomoses intestinais. Estudo experimental em coelhos [Tese]. Belo Horizonte:Faculdade de Medicina da UFMG;1993.

3. Schenk WG 3rd, Goldthwaite CA Jr, Burks S, Spotnitz WD. Fibrin sealant facilitates hesmostasis in arteriovenous polytetrafluoroethylene grafts for renal dialysis access. Am Surg. 2002;68(8):728-32.

4. Kheirabadi BS; Field-Ridley A, Pearson R, MacPhee M, Drohan W, Tuthill D. Comparative study of the efficacy of the common topical hemostatic agents with fibrin sealant in a rabbit aortic anastomosis. J Surg Res. 2002;106(1):99-107.

5. Kheirabadi BS, Pearson R, Tuthill D, Rudnicka K, Holcomb JB, Drohan W, et al. Comparative study of the hemostatic efficacy of a new human fibrin sealant: is an antifibrinolytic agent necessary? J Trauma. 2002;52(6):1107-15.

6. Jackson MR, Gillespie DL, Longenecker EG, Goff JM, Fiala LA, O'Donnell SD, et al. Hemostatic efficacy of fibrin sealant (human) on expanded poly-tetrafluoroethylene carotid patch angioplasty: a randomized clinical trial. J Vasc Surg. 1999;30(3):461-6.

7. Kheirabadi BS, Pearson R, Rudnicka K, Somwaru L, MacPhee M, Drohan W, et al. Development of an animal model for assessment of the hemostatic efficacy of fibrin sealant in vascular surgery. J Surg Res. 2001;100(1):84-92.

8. Giovannacci L, Eugster T, Stierli P, Hess P, Gurke L. Does fibrin glue reduce complications after femoral artery surgery? A randomized trial. Eur J Vasc Endovasc Surg. 2002;24(3):196-201.

9. Milne AA, Murphy WG, Reading SJ, Ruckley CV. A randomized trial of fibrin sealant in peripheral vascular surgery. Vox Sang. 1996;70(4):210-2.

10. Glimaker H, Bjorck CG, Hallstensson S, Ohlsen L, Westman B. Avoiding blow-out of the aortic stump by reinforcement with fibrin glue: a report of two cases. Eur J Vasc Surg. 1993;7(3):346-8.

11. Seguin JR, Picard E, Frapier JM, Chaptal PA. Repair of the aortic arch with fibrin glue in type A aortic dissection. J Card Surg. 1994;9(6):734-9.

12. Han SK, Kim SW, Kim WK. Microvascular anastomosis with minimal suture and fibrin glue: experimental and clinical study. Microsurgery. 1998;18(5):306-11.

13. Buijsrogge MP, Verlaan CW, Van Rijen MH, Grundeman PF, Borst C. Coronary end-to-side sleeve anastomosis using adhesive in off-pump bypass grafting in the pig. Ann Thorac Surg. 2002;73(5):1451-6.

14. Buijsrogge MP, Scheltes JS, Heikens M, Grundeman PF, Pistecky PV, Borst C. Sutureless coronary anastomosis with an anastomotic device and tissue adhesive in off-pump porcine coronary bypass grafting. J Thorac Cardiovasc Surg. 2002;123(4):788-94.

15. Fasol R, Schumacher B, Schlaudraff K, Hauenstein KH, Seitelberger R. Experimental use of a modified fibrin glue to induce site-directed angiogenesis from the aorta to the heart. J Thorac Cardiovasc Surg. 1994;107(6):1432-9.

16. Kawamura M, Sawafuji M, Watanabe M, Hourinouchi H, Kobayashi K. Frequency of transmission of human parvovirus B19 infection by fibrin sealant used during thoracic surgery. Ann Thorac Surg. 2002;73(4):1098-100.

17. Tabuchi N, Tanaka H, Arai H, Mizuno T, Nakahara H, Oshima N, et al. Double-patch technique for postinfarction ventricular septal perforation. Ann Thorac Surg. 2004;77(1):342-3.

18. Frost-Arner L, Spotnitz WD, Rodeheaver GT, Drake DB. Comparison of the thrombogenicity of internationally available fibrin sealants in an established microsurgical model. Plast Reconstr Surg. 2001;108(6):1655-60.

19. Reicher ME, Burihan E, Amorim JE, Nakano LCU, Barros N Jr, Egani MI, et al. Utilização da cola de fibrina em suturas vasculares: aspectos hemodinâmicos. Cir Vasc Angiol. 2001;17(6):195-201.

20. Taylor LM Jr. Introduction: Does the evidence justify the routine use of fibrin sealants in cardiovascular surgery? Cardiovasc Surg. 2003;11(Suppl 1):3-4.

21. Unlu Y, Vural U, Kocak H, Ceviz M, Becit N, Akbulut O. Comparison of the topical haemostatic agents for the prevention of suture hole bleeding: an experimental study. Eur J Vasc Endovasc Surg. 2002;23(5):441-4.

22. Tawes RL Jr, Sydorak GR, DuVall TB. Autologous fibrin glue: the last step in operative hemostasis. Am J Surg. 1994;168(2):120-2.

23. Kjaergard HK, Trumbull HR. Vivostat system autologous fibrin sealant: preliminary study in elective coronary bypass grafting. Ann Thorac Surg. 1998;66(2):482-6.