Table I. *In vitro* effect of hemodilution on whole blood clotting time (seconds)

Stimulus	Pathway	Degree of hemodilution			
		Controls	25%	50%	75%
Celite	Intrinsic	459 ± 75	442 ± 67	488 ± 94	951 ± 154*
Thromboplastin	extrinsic	294 ± 29	251 ± 21	277 ± 34	355 ± 61
Thrombin	common	165 ± 51	113 ± 58	91 ± 34	55 ± 19

Values are expressed as mean ± standard deviation.

*In 9 of 10 samples clotting time was more than 1000 seconds.

Table II. *Clinical observation in neonates undergoing cardiac operations*

	After heparinization	5 min CPB	30 min CPB	60 min CPB	90 min CPB	End CPB
ACTc (sec)	669 ± 233	980 ± 63*	992 ± 63*	995 ± 15*	1000 ± 0	774 ± 214
HiTT (sec)	168 ± 58	163 ± 80	132 ± 31	132 ± 53	118 ± 53	95 ± 30
Heparin (IU/ml)	2.7 ± 0.4	1.2 ± 0.5	1.1 ± 0.5	1.3 ± 0.4	1.2 ± 0.5	0.8 ± 0.3

Values are expressed as mean ± standard deviation. ACTc, Celite activated clotting time; HiTT, high-dose thrombin time; CPB, cardiopulmonary bypass.

*In 9 of 10 samples ACTc was greater than 1000 seconds.

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THE USE OF BIOLOGIC GLUE FOR BETTER ADHESIONS BETWEEN THE SKELETAL MUSCLE FLAP AND THE MYOCARDIUM AND FOR INCREASING CAPILLARY INGROWTH

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To date, dynamic cardiomyoplasty relies on use of a properly conditioned, adequately contracting autogenous muscle flap for the correction of myocardial insufficiency. Despite its advantages, cardiomyoplasty has some unresolved problems: (1) poor angiogenic potential of the ischemic, traumatized skeletal muscle and damaged myocardium and (2) incomplete contact between the mobile myocardial wall and the transplanted flap.

These problems manifest themselves after the operation. The vascular supply is not reintroduced until 6 to 8 months after the operation. Some patients have died of recurrent myocardial infarction 3 to 5 months after cardiomyoplasty, before a new blood supply from the skeletal muscle to the myocardium had been established.^{1,2} This is the situation that needs to be resolved. Cardiomyoplasty done with skeletal muscle can provide capillary ingrowth to the myocardium, but it may be too late for some patients.

A novel approach to resolving these problems involves "therapeutic angiogenesis." Nine weeks after a modified fibrin glue (containing endothelial cell growth factor) has been applied between the aorta and the left ventricular myocardium, angiographic and histologic investigations have shown newly grown vascular structures between

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Fig. 1. Macrosection 8 weeks after cardiomyoplasty. Strong adhesions (1) had formed between the latissimus dorsi muscle (2) and the myocardium (3) where the biologic glue was applied. No adhesions had formed in the area without biologic glue (4).

these sites.³ The results of this study confirm previous observations that site-directed neovessel formation can be induced by application an angiogenic growth factor.^{4,5}

We have implemented the idea of using biologic glue based on a fibrinogen-thrombin composition for cardiomyoplasty. Fibrinogen, the main component of biologic glue, was prepared from donor animals by a standard cryoprecipitation technique. In brief, whole blood obtained from the donor was centrifuged and the plasma decanted. After the plasma had been frozen for 12 hours at -80°C , it was thawed at 4°C for 4 hours and then centrifuged. The yellowish white precipitate that remained after decantation was collected and stored at -18°C . The resulting cryoprecipitate contained concentrated fibrinogen, factor XIII, fibronectin, and vitronectin. In other experiments pure fibrinogen from sheep plasma (F-9754, Sigma Chemical Company, St. Louis, Mo.) was used. Thrombin was purchased from Johnson & Johnson Patient Care, Inc. (New Brunswick, N.J.). In both cases, final solutions of fibrinogen and thrombin were prepared in separate delivery systems. After mobilization of the latissimus dorsi muscle in adult sheep, the muscle was introduced into the thoracic cavity and wrapped around the heart. The muscle flap was sutured to the myocardium of the left ventricle and two isolated pockets were created.

Fig. 2. Histologic examination obtained from a macrosection 8 weeks after cardiomyoplasty and biologic glue application showed vascular growth within the biologic glue (1). Note new blood vessels of significant diameter (5) and growth between the myocardium (3) and the latissimus dorsi muscle (2).

In one of the pockets the biologic glue was introduced by simultaneously applying the two separate solutions of fibrinogen and thrombin between the skeletal muscle and myocardium. The amount of glue used per animal was in the range of 20 to 30 ml. As a control, no biologic glue was introduced in the other pocket. After the operation the animals had the usual postoperative management and the typical stimulation protocol for skeletal muscle training.

After 8 weeks we examined the muscle flap and the myocardium of the left ventricle (Fig. 1). We saw extremely strong adhesions between the latissimus dorsi muscle and the myocardium in the pocket where the biologic glue had been applied. Histologic examination showed new blood vessel growth of significant diameter (Fig. 2). No adhesions had developed in the areas in which biologic glue was not used.

In summary, we have shown that we can induce significant formation of new blood vessel structures and produce strong adhesions between the myocardium and skeletal muscle with the use of biologic glue. This finding may be helpful for patients undergoing cardiomyoplasty.

Enhancement of angiogenesis and vasculogenesis after cardiomyoplasty might be achieved by local implantation of autologous endothelial cells cultured *ex vivo* or by application of angiogenic factors like vascular endothelial growth factor, platelet-derived growth factor, insulin-like growth factor, fibroblast growth factor, or extracellular matrix components like fibronectin, laminin, and proteoglycans. From this point of view, the fibrin meshwork serves only as a local delivery depot. However, it seems to us that a pure fibrin meshwork can sufficiently mimic the extracellular matrix for endothelial cells and support their growth. We believe that further study is required to assess the potential of various growth factors and suppressors of ischemia-reperfusion lesions in shortening the recovery period between surgery and full assistance in pumping functions of the native heart.

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