

[10, 11]. CDS can be transported to hospitals in a frozen state and stored at hospitals in a freezer. Immediately prior to clinical application, cryopreserved CDS is thawed and then rinsed with lactated Ringer's solution to remove cryoprotectant such as DMSO and FBS. According to the protocol based on our fundamental study, 412 clinical trials have been performed in the period from April 2001 to March 2005 with the support of the Millennium Project of the Ministry of Health Labor and Welfare. The results of a multi-center clinical study suggest that this type of allogeneic CDS is effective in promoting wound healing of full-thickness skin defects such as severe chronic ulcer and burn injury [12-16].

In this study, CDS cryopreserved at -85°C for 6 months or -152°C for 1 year maintained the cell viability and the ability to proliferate and to release VEGF at the same level as the control (at -152°C for 3 weeks). These findings suggest that long-term cryopreserved CDS can be used in clinical applications. Manufacturing and then banking allogeneic CDS should be established based on a practical cryopreservation schedule.

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Legends

Figure 1.

Number of cells in CDS before freezing (a); after thawing (b); after thawing followed by re-culturing for 1 week (c). n=3, mean \pm S.D.

A: Fresh CDS before freezing

B: CDS cryopreserved at -152°C for 3 weeks (control)

C: CDS cryopreserved at -85°C for 6 months

D: CDS cryopreserved at -85°C for 1 year

E: CDS cryopreserved at -152°C for 1 year

F: CDS cryopreserved at -152°C for 2 years

Figure 2.

Amount of VEGF released from CDS before freezing (a); after thawing followed by re-culturing for 1 week (c). n=3, mean \pm S.D.

A: Fresh CDS before freezing

B: CDS cryopreserved at -152°C for 3 weeks (control)

C: CDS cryopreserved at -85°C for 6 months

D: CDS cryopreserved at -85°C for 1 year

E: CDS cryopreserved at -152°C for 1 year

F: CDS cryopreserved at -152°C for 2 years

Figure 3.

Scanning electron microphotograph of CDS: atelo-collagen surface view (a), original magnification \times 80; cross-sectional view (b), original magnification \times 30

A: Fresh CDS before freezing

B: CDS cryopreserved at -152°C for 3 weeks (control)

D: CDS cryopreserved at -85°C for 1 year

F: CDS cryopreserved at -152°C for 2 years

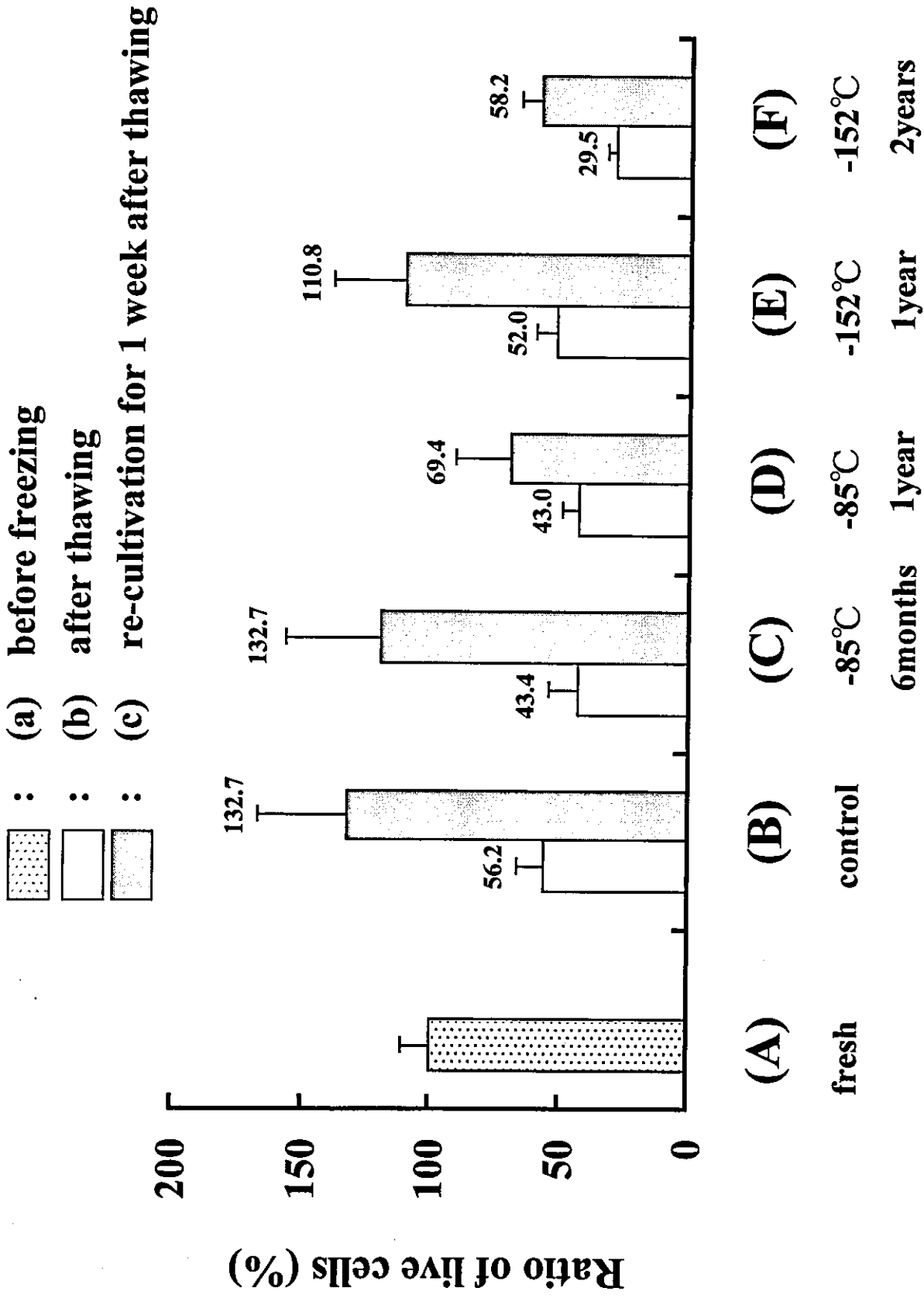


Fig.1

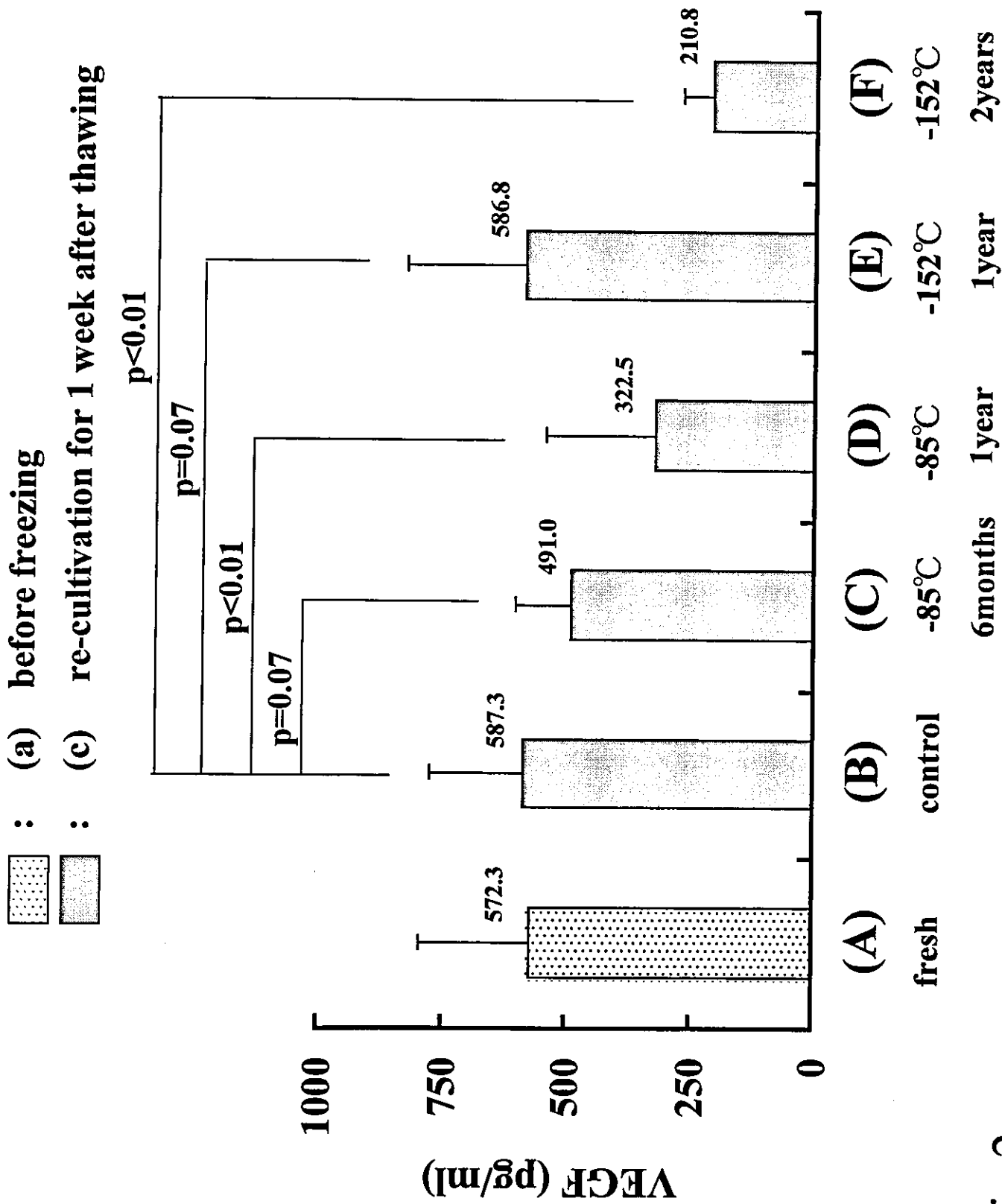


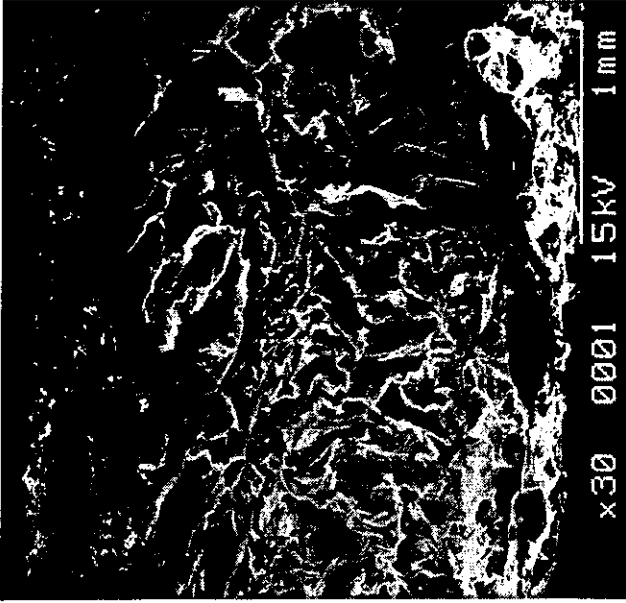
Fig.2

(b)

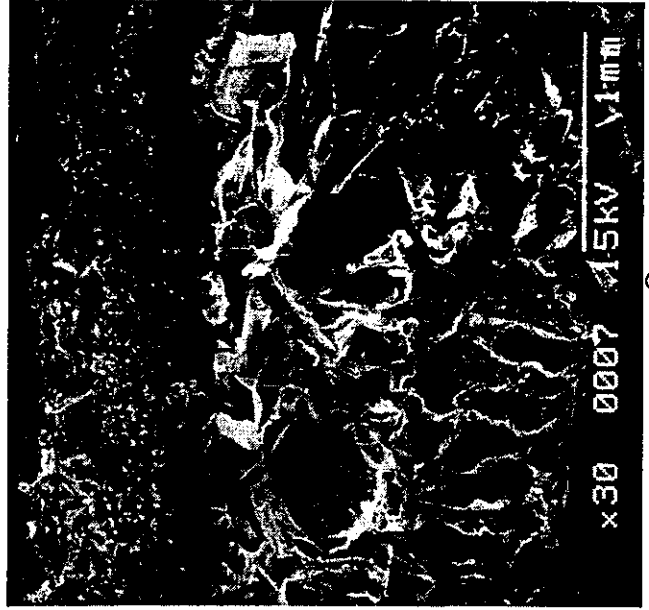
Non-woven
fabric

HA

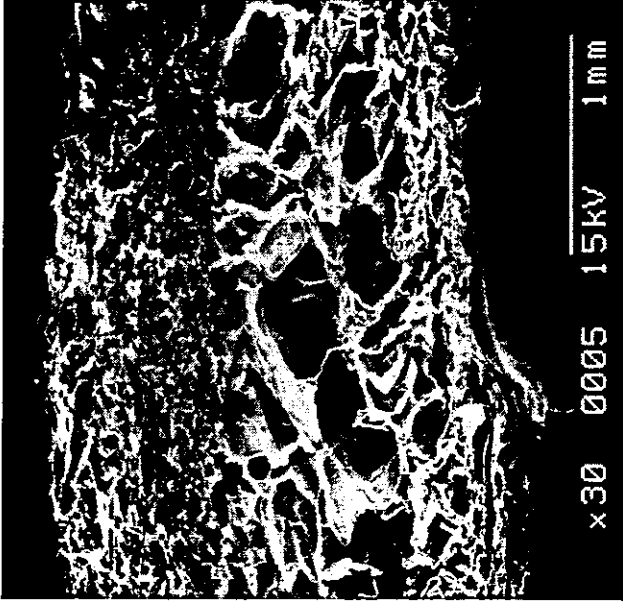
Col



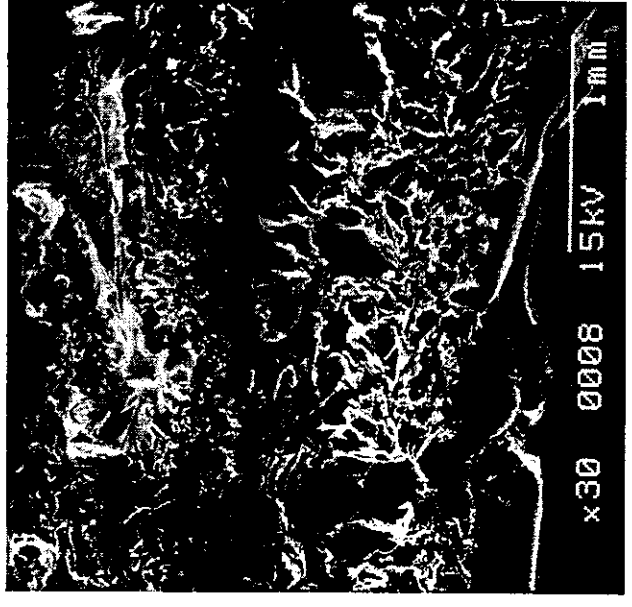
[A] fresh



[D] -85°C, 1 year



[B] control

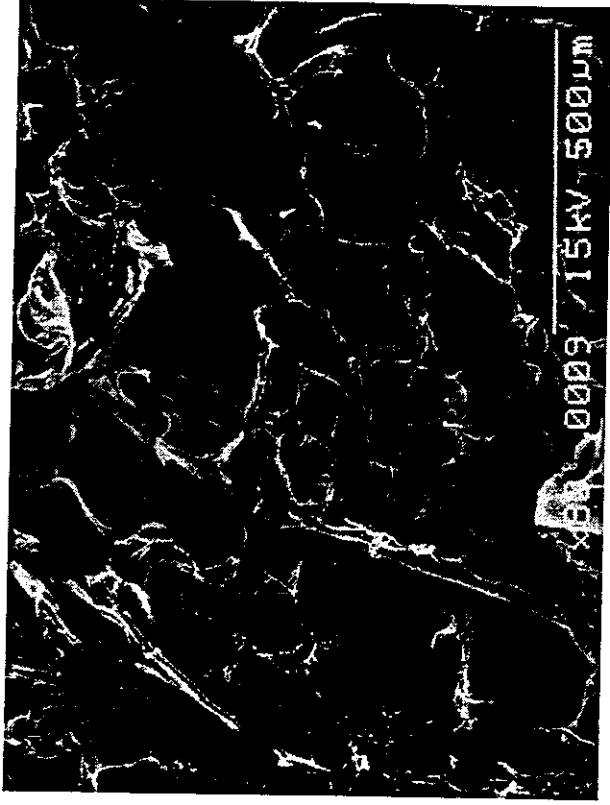


[F] -152°C, 2 years

(a)



[A] fresh



[B] control



[D] -85°C, 1 year



[F] -152°C, 2 years

Fig.3