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Treatment of Full-thickness Skin Defect with Concomitant Grafting of 6-fold Extended Mesh Auto-skin and Allogeneic Cultured Dermal Substitute

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Abstract: The aim of this clinical study was to evaluate an allogeneic cultured dermal substitute (CDS) as a biological dressing for highly extended mesh auto-skin grafting. The subjects were five patients with extensive deep burn wounds. Allogeneic CDS was prepared by seeding fibroblasts on a spongy matrix of hyaluronic acid and atelocollagen. Six-fold extended auto-skin graft was applied to the debrided wound, on which allogeneic CDS was placed. A conventional ointment-gauze dressing was used to protect the CDS. The CDS was applied repeatedly at intervals of 5–7 days. In all cases, the wounds were closed by successful take of mesh auto-skin graft and prompt epithelization from the grafted skin. The skin on the grafted area had a cicatrix appearance, but was soft and thin, maintaining good quality. The application of 6-fold extended auto-skin graft in conjunction with allogeneic CDS is an effective method for treatment of extensive severe burn wounds. **Key Words:** Cultured dermal substitute—Fibroblasts—Hyaluronic acid—Collagen—Mesh skin grafting.

A typical engineered product is autologous cultured epidermal substitute (CES), which is composed of stratified keratinocytes (1–4). There are two types of allogeneic cultured dermal substitute (CDS) composed of fibroblasts on a scaffold (5–10). Another skin substitute is allogeneic cultured skin substitute (CSS), which is composed of keratinocytes and fibroblasts on a scaffold (11–15). Recently, however, the commercialization of these allogeneic products has been discontinued. There seem to be some problems in the design of these products.

Kuroyanagi et al. (16–19) developed an allogeneic CDS composed of a spongy collagen containing fibroblasts. The efficacy of this allogeneic CDS on wound healing has been studied in animal tests and in preliminary clinical trials. On the basis of this technique, a new type of CDS was developed by culturing fibroblasts on a 2-layered spongy matrix of hyaluronic acid (HA) and atelo-collagen (Col) (20–24).

A multicenter clinical study on the use of allogeneic CDS was performed in 30 hospitals across Japan as the Regenerating Medical Millennium Project of the Ministry of Health, Labor and Welfare. Allogeneic CDS has been applied to debrided wound surfaces to prepare wound beds for split-thickness auto-skin graft, and reported results of clinical studies at other hospitals involved in this project indicate that use of CDS for this procedure has generally been successful (25–27).

Surgical closure with auto-skin grafting is the gold standard for treatment of victims with extensive deep dermal burns (DDB) or deep burns (DB). However, if the donor site is limited, an alternative treatment may be required. Although autologous CES can serve as an alternative material, its use raises practical problems, including the long preparation process (>3 weeks), the need to prepare the wound bed for CES, and reduced growth of keratinocytes derived from geriatric burn patients. The most practical treat-

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ment is mesh auto-skin grafting. Generally, a 1.5- or 3-fold extended mesh auto-skin graft is used because it usually results in successful epithelization. In practice, a mesh auto-skin graft is applied on the debrided wound surface, on which a conventional ointment gauze dressing was placed to protect the mesh auto-skin graft. When 6-fold extended mesh auto-skin graft has been applied to a wound surface in poor condition, the mesh skin graft has failed to take. With highly extended skin grafting, there is a risk of poor epithelization. To overcome this problem, excellent biological dressing is required. In the present study, we evaluated allogeneic CDS as coverage for a 6-fold extended mesh auto-skin graft.

MATERIALS AND METHODS

Preparation of spongy matrix composed of hyaluronic acid (HA) and atelo-collagen (Col)

The spongy matrix was prepared using a method described in previous articles (21–23). An aqueous solution of HA with cross-linking agent was poured into a polystyrene dish (11 × 10 cm); a sheet of hydrated cellulose nonwoven fabric was attached to the bottom of the dish. The HA solution in the dish was frozen in a freezer at –85°C and then lyophilized to obtain the HA sponge. After the sponge was rinsed thoroughly with distilled water to remove free cross-linking agent, the hydrated HA sponge was frozen and lyophilized to obtain the purified HA sponge. The purified HA sponge was punched mechanically to produce many holes. The Col solution was poured into a polystyrene dish (11 × 10 cm). The HA sponge with many holes was carefully immersed in the dish containing Col solution, with a sheet of nonwoven fabric resting on the upper side of the HA sponge, and was then frozen and lyophilized to obtain a 2-layered sponge of HA and Col. Both surfaces of the 2-layered sponge were irradiated with ultraviolet light to produce intermolecular cross-linking of Col molecules. Each sponge was then packed in a bag and kept in a dry sterilizer at 121°C for 2 h.

Establishment of cell banking

A small piece of skin was obtained from a 3-month-old patient during surgical excision of an excrescence. This patient was free from infectious viruses such as HBV, HCV, HIV, and HTLV, and results of the treponema pallidum hemagglutination test (TPHA) were negative. All procedures were in compliance with the ethical guidelines of St. Marianna Medical College. The sterilized piece of skin was immersed in Dulbecco's modified Eagle's medium (DMEM) containing dispase for 20 h at 4°C. After this enzymatic treatment, the epidermis was mechanically separated from the dermis. The dermis was minced, and was then treated with 0.5% collagenase in DMEM supplemented with 1% fetal bovine serum (FBS) for 2 h at 37°C to obtain the cellular suspension. Then, fibroblasts were cultivated in culture medium to establish cell banking. The cells were checked for viruses such as HBV, HCV, HIV, HTLV, and Parvovirus (20,22).

Preparation of cultured dermal substitute

The CDS was prepared using a method described in previous articles (22–24). Prior to seeding of fibroblasts, the 2-layered sponge of HA and Col (10.5 cm × 9.5 cm) was immersed in 50 mL of culture medium in a polystyrene dish (11 cm × 10 cm), to hydrate the sponge and neutralize its acidity. The excess culture medium was carefully removed from the dish by suction. Fibroblasts obtained from successive cultivation of the cryopreserved cells were seeded onto the 2-layered sponge, by adding 5 mL of cellular suspension dropwise onto the collagen surface of the 2-layered sponge. The number of fibroblasts on the 2-layered sponge was adjusted to 1.0×10^5 cells/cm^2. The seeded sponge was kept in an incubator in a humidified atmosphere of 5% CO_2 at 37°C overnight, followed by addition of 50 mL of culture medium and culturing for 1 week.

Fibroblasts used in production of CDS were checked for mycoplasma. The culture medium used in production of CDS was checked for bacteria.

Cryopreserving and thawing of CDS

The CDS was turned upside down in a polystyrene dish, and the culture medium was replaced with 30 mL of DMEM supplemented with 10% DMSO and 20% FBS. The CDS was frozen in the dish in a programmable freezer (AIR BLASTER, EBAC, Tokyo, Japan) at a gradient of –1°C/min from 4 to –60°C, and was then cryopreserved in a freezer at –152°C (23,24). The cryopreserved CDS (in the polystyrene dish) was placed in a foam polystyrene box containing dry ice, and was then shipped to hospitals, where it was preserved at –85°C. Prior to clinical application, the CDS (in the polystyrene dish) was placed in a foam polystyrene box at room temperature for 30 min and then floated in a water bath at 37°C, followed by rinsing with lactated Ringer's solution to remove DMSO and FBS.

Clinical evaluation

The clinical evaluation of allogeneic CDS was conducted in compliance with the ethical guidelines of

TABLE 1. *Evaluation of efficacy according to four different conditions*

	Very good	Good	Medium	Poor	Very poor
Epithelization	35	27	18	9	0
Granulation tissue	35	27	18	9	0
Control of infection	15	13	8	4	0
Drainage condition	15	13	8	4	0

Kagawa Prefectural Central Hospital. The subjects were five patients with extensive deep burn wounds and one patient with necrotizing fasciitis. Skin fragments for grafting were collected from healthy skin on the back, at a split-thickness of 0.010–0.012 in. Six-fold extended mesh auto-skin graft was applied to the debrided wound, and was fixed using a stapler, with allogeneic CDS placed over the wound. A conventional ointment-gauze dressing was used to protect the CDS. The CDS was applied repeatedly at intervals of 3–5 days.

After treatment of full-thickness skin defects with concomitant grafting of 6-fold extended mesh auto-skin and allogeneic CDS, we clinically evaluated epithelization, granulation tissue formation, control of wound infection, and drainage conditions. Epithelization and granulation tissue formation were graded according to the following scale: very good, 35 points; good, 27 points; fair, 18 points; poor, 9 points; very poor, 0 points. Drainage conditions and control of wound infection were graded according to the following scale: very good, 15 points; good, 13 points; fair, 8 points; poor, 4 points; very poor, 0 points (Table 1). Safety was graded according to the following scale: A, very safe; B, mostly safe; C, problem with specific treatment; D, not safe.

Total evaluation consisted of judging both efficacy and safety (Table 2). Cases with a total point score >80 and a safety grade of A were assessed as excellent. Cases with a total point score ≥60 and ≤79 and a safety grade of A or B were assessed as good. Cases that fulfilled either of the following sets of criteria were assessed as fair: total point score ≥40 and ≤59, and safety grade of A, B, or C; total point score >60, and safety grade of C. Cases that fulfillled either of the following sets of criteria were assessed as poor: total point score ≥20 and ≤39, and safety grade of A, B, C, or D; total point score >40, and safety grade of D (26–28).

CASE REPORTS

Three representative cases are presented below.

Case 1: An 81-year-old female was injured when her clothes caught fire while she was burning dry grass as part of farm work. The injury was located in the lumbar-gluteal region over the bilateral lower limbs, and a mixture of DDB and DB accounted for 36% of the injured region. On day 6, when her general condition had improved, debridement of both lower legs was performed up to the fat layer, and 6-fold extended mesh auto-skin fragments were grafted onto the right lower leg, followed by application of allogeneic CDS (Fig. 1A–C). On day 13, debridement of the bilateral femoral and gluteal regions was performed up to the fat layer, and 6-fold extended mesh auto-skin fragments were grafted onto the left femoral region, followed by application of allogeneic CDS. Take and epithelization of the grafts were good on day 18 after grafting on the right lower leg (Fig. 1D) and on day 14 after grafting on the left femoral region (not shown). The application of CDS was continued for a period of 42 days at both sites. The patient had mild dementia and rejected bathing, but the epithelialized wound area seemed to itch less than the regions treated with conservative therapy, and good cicatrices were formed without scratch wounds. The patient was discharged wheelchair-bound 9 months after surgery, after gait training.

Case 2: A 79-year-old male fell into a bonfire and was burned. The injury was a mixture of DDB and DB accounting for 25% of the back, gluteal regions and both hands. On day 6, when the general condition had improved, debridement was performed on the bilateral gluteal regions up to the fat layer, and 6-fold extended mesh auto-skin fragments were grafted, followed by application of allogeneic CDS (Fig. 2A,B). Take and epithelization of the grafted skin were good on day 8 after grafting (Fig. 2C). Epithelization advanced smoothly on the granulation tissue under the CDS and is shown on day 22 after grafting (Fig. 2D). The application of CDS was continued for a period of 24 days. The patient was discharged 2 months after surgery, and he rides a motorcycle and visits the outpatient office once every few months. At 10 months after surgery, the cicatrices were thin and soft, maintaining good condition.

TABLE 2. *Total evaluation by judging both efficacy and safety*

Total evaluation	Efficacy	Safety
Excellent	80–100	A
Good	60–78	A or B
Fair	40–59	A, B, or C
	>60	C
Poor	20–39	A, B, C, or D
	>40	D

A, very safe; B, safe; C, with specific treatment; D, not safe.

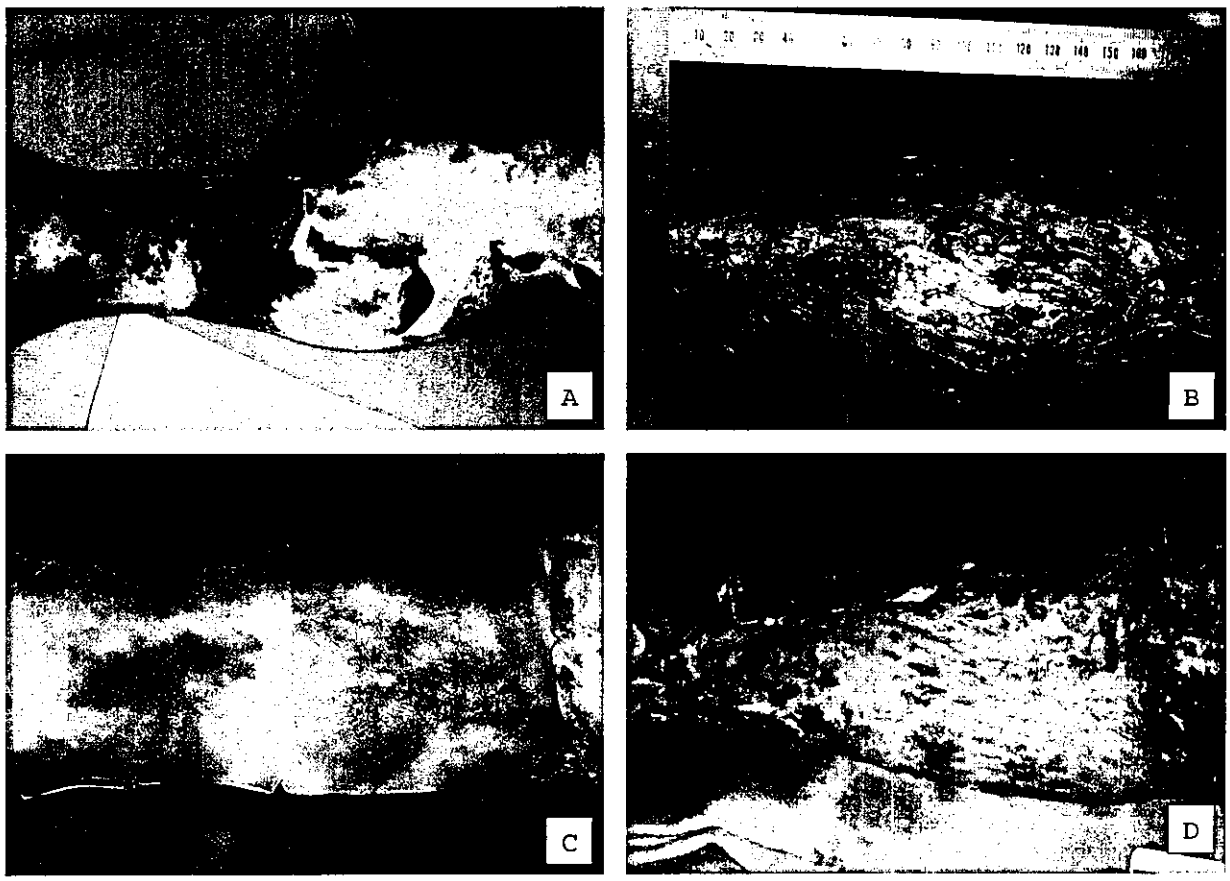

FIG. 1. Case 1: an 81-year-old female (Patient 1). The patient suffered DDB and DB on the right lower leg (A). Six-fold extended mesh auto-skin fragments were applied to the debrided wound (size 18 × 9 cm) (B), and followed by application of allogeneic CDS (C). The mesh skin took successfully and the area between strips of mesh skin were epithelized on day 18 after grafting (D).

Case 3: An 88-year-old female suffering from necrotizing fasciitis on the back lumbar region (size 39 × 28 cm) (Fig. 3A). Necrocectomy was performed up to the fat layer, and 6-fold extended mesh auto-skin fragments were grafted onto the debrided wound, followed by application of allogeneic CDS (Fig. 3B,C). Take and epithelization of the grafted skin were good on day 28 after grafting (Fig. 3D). The cicatrices were thin and soft, indicating good condition.

RESULTS

The mean duration of use of cultured allogeneic dermal substitute was 36 days, and the mean number of CDS exchanges was 7 (Table 3). In one case (Patient 4), the grafted area gave rise to infection at day 14 after grafting. Take of grafted skin was complete in all cases, and epithelization advanced smoothly in 6 cases. About 5 days after grafting, epithilization began in the area surrounding the grafted skin. Formation of good red-colored granulation between strips of mesh skin was smooth and flat, and epithelization advanced smoothly on that granulation tissue under the CDS. Healing was not slower than that of the 3-fold extended mesh skin grafts in the same patient. The duration of postoperative follow-up was 10–14 months, and cicatrices were soft and elastic. Stiffness was mild and did not disturb motor function.

DISCUSSION

A multicenter clinical study of allogeneic CDS has been conducted in 30 hospitals across Japan as part of the Regenerating Medical Millennium Project of the Ministry of Health, Labor and Welfare. This clinical study was designed to evaluate allogeneic CDS for treatment of severe wounds, including burn wounds (DDB, DB), skin ulcers, traumatic skin defects, and excise wounds from removal of giant pigmented nevus (26–28). The department of plastic reconstructive surgery of Kagawa Prefectural Central Hospital is a member of this project. In our

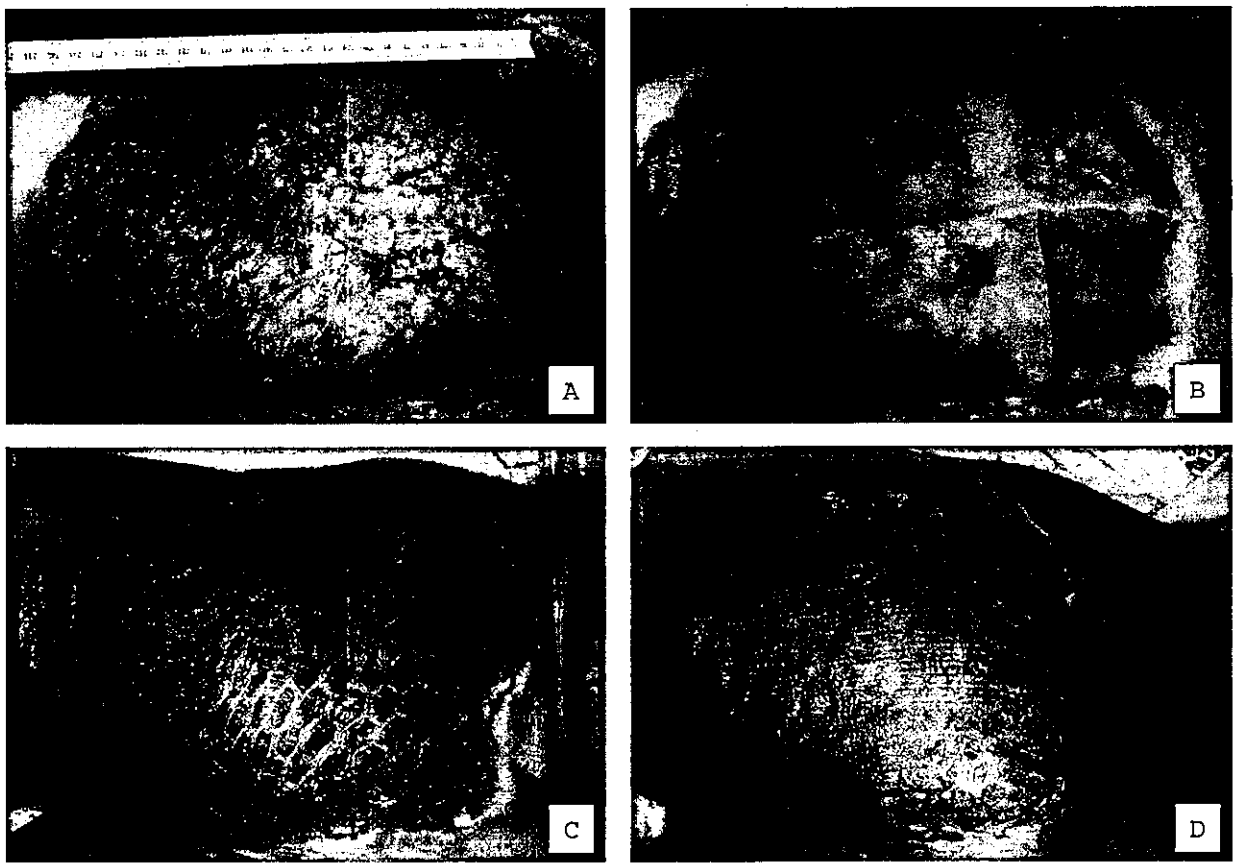

FIG. 2. Case 2: a 79-year-old male (Patient 2). The patient suffered intermingled DDB and DB on the bilateral gluteal regions. Debridement was applied up to the fat layer, and 6-fold extended mesh auto-skin fragments were grafted on the wound (A), on which the allogenic CDS was placed (B). On day 8 after grafting, the grafted skin took successfully (C). On day 22 after grafting, epithelization was advanced (D).

hospital, the focus of this clinical study was application of CDS for coverage of 6-fold extended auto-skin graft.

Allogeneic CDS fails to take permanently on the wound surface, but is able to produce cell growth factors (e.g., vascular endothelial growth factor [VEGF]) and extracellular matrix components (e.g., fibronectin), which are necessary for wound healing (22,25). The constituents of the spongy matrix of CDS promote wound healing. Hyaluronic acid has a high capacity for hydration, is involved in adherence/detachment of cells and substrates, and

TABLE 3. *Information on patients given concomitant grafting of 6-fold extended mesh auto-skin and allogeneic CDS*

	Patient 1	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age	81	81	79	39	44	54	88
Sex	M	M	M	F	M	M	F
Indication	DDB/DB	DDB/DB	DDB/DB	DDB/DB	DDB/DB	DDB/DB	Necrotiz. fasciitis
Region	Right lower leg	Left femoral region	Left gluteal region	Right forearm	Right left arm	Right lower leg	Left lumbar region
Size (cm × cm)	18 × 9	15 × 9	24 × 17	14 × 7 7 × 6	30 × 10 40 × 10	27 × 10	27 × 13
Duration (days)	42	42	22	46	28	26	47
Exchange (number)	8	7	6	7	7	7	7
Efficacy	96	96	98	88	75	82	88
Safety	A	A	A	A	C	A	A
Total evaluation	Excellent	Excellent	Excellent	Excellent	Fair	Excellent	Excellent

CDS, cultured dermal substitute; DDB, deep dermal burns; DB, deep burns.

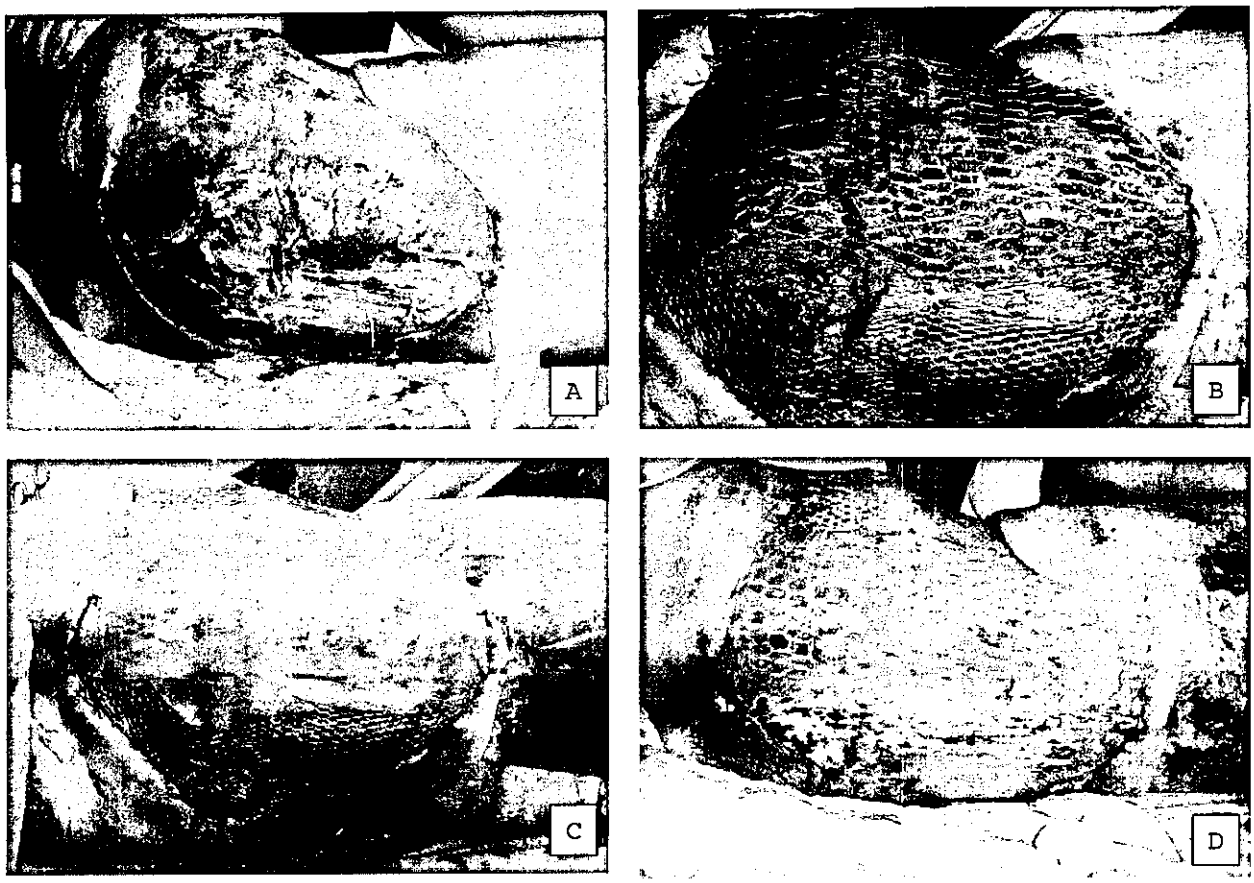

FIG. 3. Case 3: An 88-year-old female (Patient 6). The patient suffered necrotizing fasciitis on the back lumber region (A). Necrosectomy was performed, and 6-fold extended mesh auto-skin fragments were grafted (B), on which the allogeneic CDS was placed (C). On day 28 after grafting, the grafted skin took successfully and epithelization was advanced (D).

promotes cell migration (29,30). Collagen-derived polypeptide acts as a chemotactic factor for fibroblasts (31).

Treatment of deep burn wounds requires surgical closure with auto-skin grafting. However, in cases of massive burn injury, the donor area is insufficient. To reduce the area of donor skin surface needed, the area of harvested split-thickness skin can be increased by preparing mesh skin grafts. Although mesh skin grafting has disadvantages with regard to functional and esthetic aspects of cicatricial skin, it is a good method for closing wounds at an early stage, to improve the general condition of the patient. It seems that successful application of mesh skin grafting extended to more than 3-fold is difficult, because of insufficient epithelization between the strips of meshed skin. Successful application of highly expanded mesh skin grafting requires excellent biological dressing, which can promote granulation tissue formation and epithelization on the resulting granulation tissue. The present study was designed to evaluate the ability of allogeneic CDS to promote granulation tissue formation and epithelization when used as a biological dressing for 6-fold extended mesh auto-skin grafting. In all present cases, the 6-fold extended mesh auto-skin took successfully and most of the wound surface between the strips of meshed skin epithelialized within 3 weeks. In addition, the quality of final cicatrices was good. The present results suggest that allogeneic CDS is highly suitable as a biological dressing for 6-fold extended mesh auto-skin grafting. This application has promise as a therapeutic method for patients with massive severe burn injuries.

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