Correlation between Ageing and Collagen Gel Contractility of Human Fibroblasts

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To investigate the influence of ageing on wound healing, we cultured fibroblasts derived from human dermis in type I collagen gel, and evaluated the relationship between gel contractility and ageing. Cells were obtained from children (0–15 years old, Group A), early adulthood (16–40 years old, Group B), mid-adulthood (41–60 years old, Group C), and the elderly (61 or older, Group D). Gel contractility was determined by measuring the diameter on the second day after gel preparation. Within the tenth passage, gel contraction was the most marked in Group A, but did not differ among the other groups. Gel contraction at passages 30–40 did not differ from those within the tenth passage in Groups B, C and D, but it decreased markedly in Group A to a value similar to that in the other groups. These results show that fibroblasts in childhood are more contractile than those in adulthood and are moré readily affected by passages (in vitro ageing).

(Accepted November 13, 1989)

Acta Derm Venereol (Stockh) 1990; 70: 241–244.

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Wound healing is empirically known to occur earlier in younger patients and is delayed with age (1–3). In addition, studies with various in vivo experimental systems have shown that wound contracture, an important function in wound healing, decreases with age (2). However, the mechanism still remains obscure.

Bell et al. (4) embedded and cultured fibroblasts in type I collagen gel and observed formation of a dermis-like structure following gel contraction. They suggested that this gel contraction phenomenon could be an in vitro model of wound contracture.

We evaluated the collagen gel contractility of fibroblasts derived from human dermis at various ages and also investigated the contractility at early passages and late passages of culture (in vitro ageing).

MATERIALS AND METHODS

Cell culture

Human dermal fibroblasts were obtained from explants of normal dermis of variously aged men and women as described previously (5). All cells were grown and maintained in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Tokyo, Japan) containing 10% (V/V) fetal

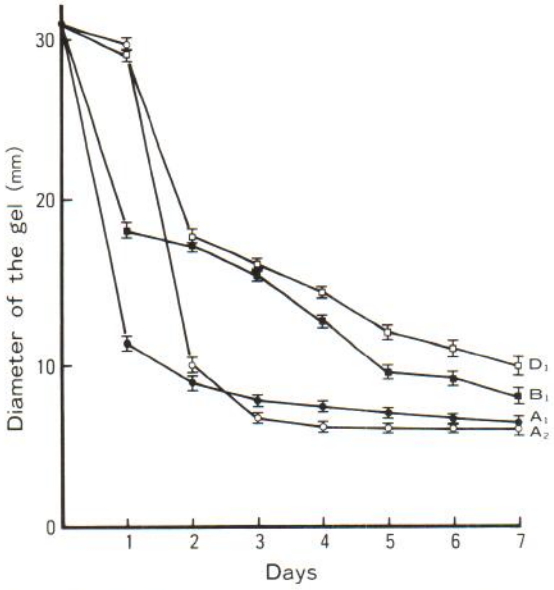

Fig. 1. Time course of collagen gel contraction by embedded fibroblasts of representative samples in each age group. Each dot represents the mean of three gels (± SD).

calf serum (FCS) (GIBCO, Grand Island, NY) in 75 cm^2 tissue culture flasks (Corning).

Experimental groups

Cell strains used were classified into the four groups specified below, according to the donor's age. Six strains were assigned to each group.

A: Children (0–15 years of age, mean 10 ± 2.5 years)
B: Adolescent (16–40 years of age, mean 27 ± 7.3 years)
C: Middle aged (41–60 years of age, mean 51 ± 5.6 years)
D: Elderly (over 61 years of age, mean 68 ± 4.9 years)

They were used during relatively early passages [within 10 population doubling levels (PDLs). When they were aged in vitro (late passages), their signs were changed to A', B', C' and D', respectively.

Preparation of collagen gels

Prior to use, the cells were treated briefly with an 0.25% trypsin (GIBCO) solution, washed twice, counted and diluted with medium to the desired concentrations.

The fibroblasts were embedded three-dimensionally in hydrated collagen gels using a modified version of the procedure developed by Elsdale & Bard (6). The following stock solutions were prepared and kept below 4°C. Solution I: 0.3% (W/V) pepsinized type I collagen solution in dilute HCl (pH 3.0) (derived from porcine tendon and checked for purity by SDS-PAGE, Nitta Gelatine Co., Osaka, Japan); Solution II: Dulbecco's modified Eagle's medium concentrated five-fold; and Solution III: 200 mM HEPES in 0.08 N NaOH. Seven volumes of Solution I, two volumes of Solution II were mixed with one volume of Solution III and FCS in plastic tube (collagen mixture). After the suspension containing the appropriate number of cells (1/10 volume of total collagen mixture) was added to the tube, 1 ml aliquots of this mixture were poured into 35-mm bacteriological dishes (Falcon), which were incubated at 37°C. The collagen gelled within 10 min and the cells were trapped three-dimensionally within the gels. The final collagen concentration was 2 mg/ml. The initial cell density was 10^5 cells/ml.

Measurement of gel size

Gels diameters were measured every 24 h by placing the dishes over a ruler. Mean diameters in three directions were recorded. The significance of difference in mean diameters was determined by paired or non-paired Student's *t*-test.

RESULTS

Time course of collagen gel contraction

The time-related changes in the diameter of collagen gel in representative samples in each age group (A_1, A_2, B_1, D_1) are shown in Fig. 1. Contraction began within 1–2 days of gel preparation and continued. The diameter 7 days after gel preparation was 25–37.5% of the original value. As the gradient of each curve shows, contraction rates were the greatest until 2 days after gel preparation. After one day, A_1 decreased to 38% and B_1 to 59%, but contractions of A_2 and D_1 were slight. After 2 days, A_2 and D_1 also decreased markedly to 31% and 58%, respectively.

All the samples used in this study showed similar time courses with the initiation of marked contrac-

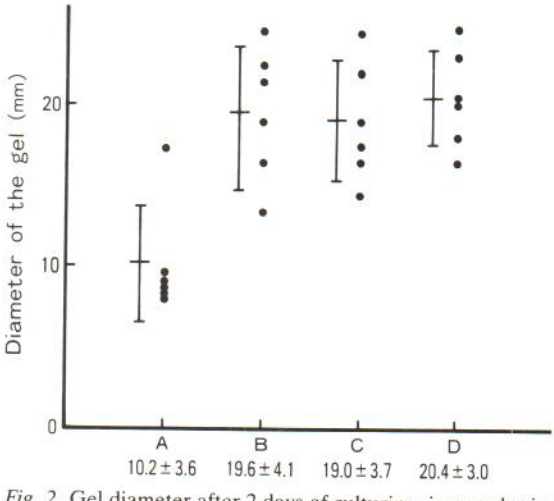

Fig. 2. Gel diameter after 2 days of culturing, in samples in each age group following relatively few passages (within ten PDLs). Each dot represents the mean of three gels. Bar at left side of the dots indicates mean ± SD.

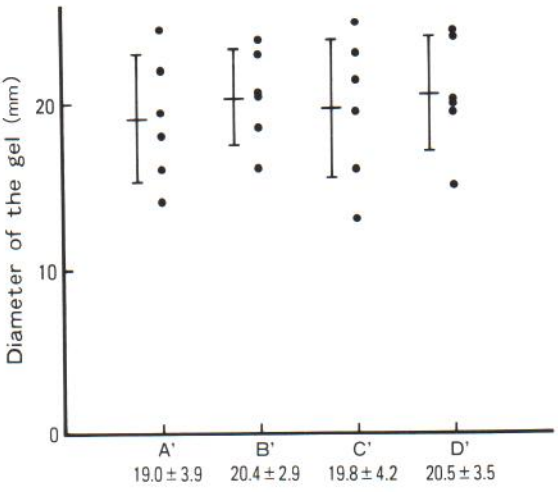

Fig. 3. Gel diameter after 2 days of culturing, in samples in each age group following relatively many passages (30–40 PDLs, in vitro ageing). Each dot represents the mean of three gels. Bar at left side of the dots indicates mean ± SD.

tion within 1–2 days of gel preparation. No sample showed rapid contraction after 3 days. Until after about 6 days, the gel was circular but gradually became irregular, making accurate measurements of the diameter difficult. Therefore, in the experiments below, gel diameters were compared after 2 days, when the most pronounced change was observed in all gel samples.

Relationship between gel contractility and age

Fig. 2 shows the gel diameter after 2 days in samples in each group following relatively few passages (within ten PDLs). In the children's group (A), gel diameters were 10.2 ± 3.6 mm, being significantly smaller than in the other groups ($p < 0.01$). The gel diameters in the early adulthood (B), mid-adulthood (C), and elderly (D) groups were 19.6 ± 4.1, 19.0 ± 3.7 and 20.4 ± 3.0 mm, respectively, showing no significant differences.

Relationship between gel contractility and in vitro ageing

Fig. 3 shows gel diameters after 2 days in samples in each group following serial passages (30–40 PDLs, in vitro ageing). Gel diameters (mm) were 19.0 ± 3.9 in the children's group (A'), 20.4 ± 2.9 in group (B'), 19.8 ± 4.2 in group (C') and 20.5 ± 3.5 in group (D') (non-significant differences).

These values were compared with those in the low PDLs (Fig. 2). In the children's group (A and A'), gel diameters after 2 days increased about 1.9-fold following passage, showing marked decreases in gel contractility ($p < 0.001$). No differences were observed in gel diameter among the early adulthood (B and B'), mid-adulthood (C and C') and elderly (D and D') groups, showing no effects of passage on gel contractility.

DISCUSSION

In some in vivo and in vitro experimental systems, biological and biochemical changes with ageing have been reported, affecting wound contracture (2), tensile strength (7), collagen deposition (8), re-epithelialization (9), outgrowth and cell proliferation (10, 11).

Bell et al. (4) considered collagen gel contraction to be an in vitro model of wound contracture. Steinberg et al. (12) and Ehrlich et al. (13) demonstrated low gel contractility by fibroblasts derived from patients in whom wound healing was delayed. These observations have suggested a relationship between the gel contraction phenomenon and wound healing.

Within 10 PDLs, gel contractility was very marked in the children's group compared with the other age groups (Fig. 2). This finding may be related to very rapid wound healing in children, which is common knowledge (1, 2). On the other hand, no significant difference was observed between the adolescent and older groups.

Hayflick & Moorhead (11) reported, following ageing in vitro by serial passage, decreases in proliferation in 30–40 PDLs and no mitosis thereafter (Hayflick's law). Therefore, we performed the same experiments on cells in 30–40 PDLs. The appreciable gel contractility in children observed in the early passages was lost with in vitro ageing, and, in addition, the degrees of contractility became almost the same as those of the older groups (Figs. 2, 3). In contrast to the children's group, no significant difference was observed in contractility between those in the early and late passages among the early adulthood and the older groups (Figs. 2, 3). In other words, the gel contractility of adult cells seems to be more resistant to in vitro ageing. Bell et al. (4) also reported no change in contractility between 19 PDLs and 35 PDLs. These results are consistent with ours, though they did not mention the donors' ages.

Earlier wound healing in children may be explained by the highly effective contraction at the site

of the wound, demonstrated by the marked gel contractility of fibroblasts. However, the delay in wound healing in adulthood or older age groups cannot be explained by differences in the gel contractility of fibroblasts alone. Concerning the rates of wound healing at these ages, the re-epithelization process might be more important (9).

The cause of the difference in gel contractility between the children and the older groups is not clear. It is possible that certain radical changes in cell functions occur in the growth process from childhood to adulthood, such as changes in responsiveness to growth factors (3) or glycosaminoglycan synthesis (14). Moreover, changes in contractility by serial passage, observed in the children's group, may be due to in vitro ageing with serial passage (11). However, another explanation may be that cultured cells gradually lose their characteristics due to lack of stimulus from the organism. These theories warrant further investigation, since the mechanism of collagen contraction is still obscure (15).

In summary, the relationship between collagen contraction and ageing was demonstrated. Collagen gel contraction has been shown to be a useful experimental system in studies on ageing and wound healing.

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