EFFECT OF HYALURONATE ON COLLAGEN PROPERTIES

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Two types of collagen fillers have been used to improve dermal defects. A gel type with preformed collagen fibrils crosslinked (Zyderm) was initially developed, followed by a solution type with highly concentrated collagen which forms fibrils after injection into dermis (Koken). Biological stability after injection is expected to be higher for the gel type, while the penetration of collagen filler into tissues with defects is better for the solution type. We have examined the effect of hyaluronate (HA) on the physical and biological characteristics of the solution type of collagen filler.

MATERIALS AND METHODS

Two different preparations of atelocollagen (Koken) from bovine skin were used with HA (Mw.: 340k, 1200k, 1400k, 1900k or 2000k, Shiseido). Collagen fibril formation was monitored by turbidity at 450 nm. Thermal stability of the collagen aggregates was examined with a differential scanning calorimeter. Injected sites of rat back skin were examined histologically or immunohistologically with anti-bovine type I collagen specific antibody.

RESULTS AND DISCUSSION

The rate of fibril formation depended on the collagen preparations: one preparation (F) reached a turbidity half of the plateau by 1 h, but the other (S), after 55 h. The addition of HA accelerated fibril formation of particularly the collagen preparation (S) in a HA concentration dependent manner (Fig.1). The molecular weights of HA did not change the effect. The thermal stability of the collagen aggregates comprised two fractions; 60% with a lower transition temperature (42°C) and 40% with a higher one (49°C) (Fig.2-1). The aggregates formed from the mixture solution of collagen and HA showed virtually only the high transition temperature (Fig.2-2), indicating that HA caused less stable collagen aggregates to change into the more stable aggregates. In the histological analysis of collagen fillers injected into the rat dermis, the addition of HA further augmented penetration of the collagen into distant regions of the tissue. The findings were immunohistologically confirmed. One important observation is that noninflammatory cells penetrated
the reconstituted collagen fibrils, but not the preformed, crosslinked collagen fibrils. The timing of the cell appearance in the reconstituted collagen fibrils in dermis was retarded in the case of the collagen preparation(S) (Fig. 3-1). The addition of HA restored the retarded cell appearance (Fig. 3-2). This effect of HA on the preparation (S) might be related to that of HA on the fibril formation in vitro. If this is true, the appearance of noninflammatory cells in the solution filler but not in the gel filler may depend on the fibrillar architecture of the reconstituted collagen fibrils or on the fibrillar plasticity that the reconstituted collagen fibrils have in interaction with fibroblastic cells. The characteristic interactions of fibroblasts with collagen fibrils in gel culture have been well documented.

![Fig.1. Time Course of Collagen Fibril Formation.](image1)

![Fig.2. Melting Curve of collagen Aggregates by Differential Scanning Calorimetry.](image2)

![Fig.3. Histological Appearance of Collagen Fillers Injected into Rat Dermis.](image3)