Recent development in a hybridization of cells with collagen
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There has been an increasing interest in artificial organs or tissues which are reconstituted from dissociated functional cells both in the area of basic and applied researches of biology. This reconstitute has been called in several ways, like a cell-incorporated artificial organ, an organ or tissue equivalent, a test tube organ, or a cultured organ. Several lines of evidence have been reported that indicate the cells in the reconstitute behave as they do in vivo and therefore, they seem to be normal in the proliferation and the expression of differentiated functions as compared to cells in conventional culture. Accordingly, the reconstitute provides a unique and useful in vitro experimental system for studying cell morphology and physiology. The reconstitute is attracting also clinicians, because this tissue or organ equivalent can be used as a tissue or organ substitute for patients with the irreversibly damaged tissue or organ.

Tissues in animals contain extracellular matrices (ECM) as well as cells. Therefore in reconstruction of the tissue equivalent, it has been realized that ECM is an inevitable component of the reconstitute. Therefore, in trying to reconstruct a tissue-equivalent, several methods have been developed for the hybridization of cells with ECM. Here, I would like to focus on collagen as one of several ECMs and summarize studies developed in the author's laboratory on collagen to cell interactions which will provide useful informations on the methods of reconstruction of a tissue equivalent.

(1) The form of existence of collagen greatly influences the morphology and physiology of cells.
There are at least three forms of collagen; denatured, native but monomeric, and polymeric. We investigated how these forms of collagen affect the morphology and physiology of cells when cells are cultured on a substratum of collagen. Three different kinds of cells were examined: human dermal fibroblasts, rat hepatic parenchymal cells and rat epidermal cells. Almost the same conclusions have been obtained for these cells. The cells cannot recognize the differences between denatured collagen and a native, but monomeric form of collagen (hereafter, non-fibrous form) but they do recognize differences between the non-fibrous form and polymeric (fibrous) form of collagen. The former form enhances cellular spreading and induces less cytoplasmic protrusions. The rate of DNA synthesis of the cells is stimulated on non-fibrous collagen. On the contrary to this, the fibrous collagen manifests the opposite effects on the cells: suppression of cellular spreading, more cyto-
plasmic processes and depressed DNA synthesis. Fibrous collagens induce and stabilize the differentiated functions of epithelial cells (keratinization for epidermal cells and secretion of albumin for hepatocytes) as compared to non-fibrous collagens.

(2) Cells recognize differences between non cross-linked and cross-linked collagen fibrils by changing their shape and rate of DNA synthesis.

Incubation of freshly prepared collagen fibrils at 37°C for more than several days induces a non-enzymatic cross-linking among collagen fibrils. This cross-linking is markedly accelerated by the presence of reducible sugars, indicating the involvement of the Maillard's reaction in the cross-linking process. Comparative studies of behaviors of epidermal cells and fibroblasts on the non cross-linked and cross-linked collagen fibrils revealed that these cells spread much better and synthesize DNA more rapidly on the cross-linked one than on the counterpart. Furthermore, in the case of epidermal cells, they make larger colonies on the former collagen than on the other.

(3) Mechanism of collagen to cell interactions: possibility of direct interactions of cells to collagen through putative collagen receptors.

How do cells recognize the various forms of collagen and respond to them as described above? To approach for an answer, we investigated the mode of interaction between cells and collagen, by reconstituting a dermal equivalent according to E. Bell. The dermal equivalent was prepared by incorporating fibroblasts into three-dimensional lattices of collagen fibrils. In this model tissue, fibroblasts make intense interactions with collagen by contracting fibrils toward them, which results in a reduction of volumes of collagen gels. We utilized this gel contraction for quantitative determinations of the extent of cell to collagen interactions. Several lines of evidence indicate that cells interact directly with collagen, not being mediated through fibronectin. We are now investigating the nature of collagen receptors on plasma membranes, which will be helpful to solve the question described above at a molecular level.

(4) Hybridization of cells with collagen.

There are two types of hybridization of cells with collagen: in vivo hybridization and in vitro one. When we need cover the body surface with materials containing collagen or implant them inside the body for clinical purposes, we have to consider the in vivo hybridization. The reconstitution of a tissue equivalent mentioned above is an in vitro hybridization. In both cases, when we face to make the cell to collagen hybridization, it is necessary to take account of cell to collagen interactions specified above. Especially, the method of cross-linking of collagen is important clinically because collagen should be donated the physical strength and structural stability.
There have been known several chemical cross-linkers of proteins: glutaraldehyde, formaldehyde, hexamethylene diisocyanate, and polyepoxy compound (polyglycerol polyglycidyl ether). As mentioned above, cross-linking of collagen molecules greatly affects physiology and morphology of cells. However, there have been few systematic studies on the interactions of cells with chemically cross-linked collagen. We cross-linked collagen with different types of cross-linkers as listed above and compared them with respect to the response of fibroblasts in vivo and in vitro. Fibroblasts showed good responses in vitro with respect to shape and growth to collagens cross-linked by formaldehyde, hexamethylene diisocyanate and polyepoxy compound, but not by glutaraldehyde which showed some toxic effects on the cells. These collagens were chemically cross-linked in the fibrous form. However, fibroblasts did respond in the same way as they did to the non-fibrous collagen with respect to growth, while the cells showed appearance identical to that on fibrous collagen.

Cross-linked collagen sponges were subjected to the in vivo hybridization by implanting them subcutaneously to rats. In this study, cross-linkings were introduced by hexamethylene diisocyanate. Two weeks later, sponges were examined histologically. Few fibroblasts invaded the sponge. The penetration of fibroblasts into sponges were remarkably improved by either of two procedures: by the heat-denaturation of the cross-linked collagen or by coating the surface of the cross-linked sponges with heat-denatured collagen. This result supports the idea described above that denatured collagen gives fibroblasts more rapid proliferation than native collagen.

References