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Development of collagen vitrigel useful for tissue regeneration

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The fibril density of traditional collagen gel (Fig. 1) is quite low in comparison to that of connective tissue *in vivo*. Therefore, we aimed to develop a novel collagen scaffold with high fibril density. In addition, it was known that opaque egg white could be converted into a thin, transparent, rigid glass-like material by boiling and evaporating the moisture — a phenomenon known as vitrification of denatured proteins. We applied this vitrification technology to a traditional collagen gel (Fig. 1). Collagen vitrigels with high fibril density were successfully prepared by a three-step process: a gelation step in which a type-I collagen sol forms an opaque and soft gel by incubation at 37C; a vitrification step in which the gel becomes a rigid material after sufficient drying; and a rehydration step with supplemental moisture that converts the vitrified material into a thin and transparent gel membrane with enhanced gel strength. In this report, we define a vitrigel as a gel in a stable state produced by rehydration following vitrification of a traditional hydrogel. The collagen vitrigel obtained possesses excellent protein permeability (Fig. 1).

Furthermore, we also prepared framework-embedded collagen vitrigel scaffolds by inserting a nylon membrane ring into the collagen sol prior to the gelation step (Fig. 2). Anchorage-dependent cells can be cultured on both surfaces of the scaffold by the manipulation of two-dimensional cultures, resulting in the reconstruction of a three-dimensional organoid (Fig. 2). In studies of a crosstalk model between PC-12 pheochromocytoma cells and L929 fibroblasts, we found that nerve growth factor secreted from L929 cells passed through the collagen vitrigel scaffold and induced the neurite outgrowth of PC-12 cells by its paracrine effect (Fig. 3). We also found that a collagen vitrigel scaffold containing vascular endothelial growth factor (VEGF) showed a sustained release of VEGF in vitro and that its subcutaneous transplantation into a rat resulted in remarkable angiogenesis (Fig. 4). These data suggest that the collagen vitrigel scaffold is useful for paracrine assays *in vitro* and drug delivery

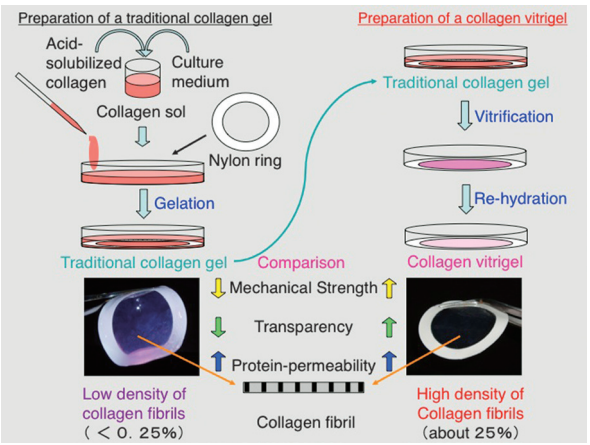

Fig. 1 Preparation methods of a traditional collagen gel and a novel collagen vitrigel and comparison of their properties

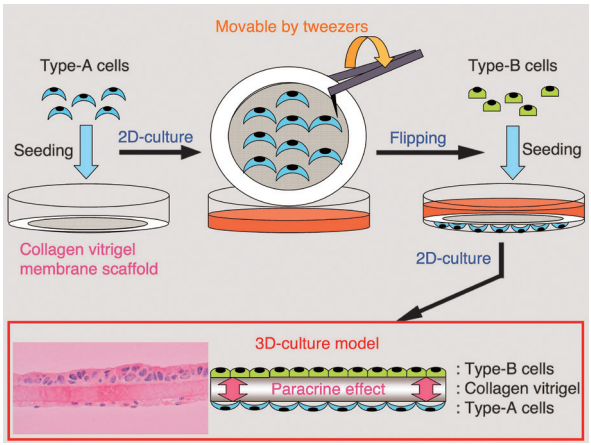

Fig. 2 Advantages of the culture technology utilizing a support material-embedded collagen vitrigel membrane scaffold

systems *in vivo*. Therefore, we expect that collagen vitrigels could contribute to studies in regenerative medicine and drug development and could serve as an alternative to animal experiments.

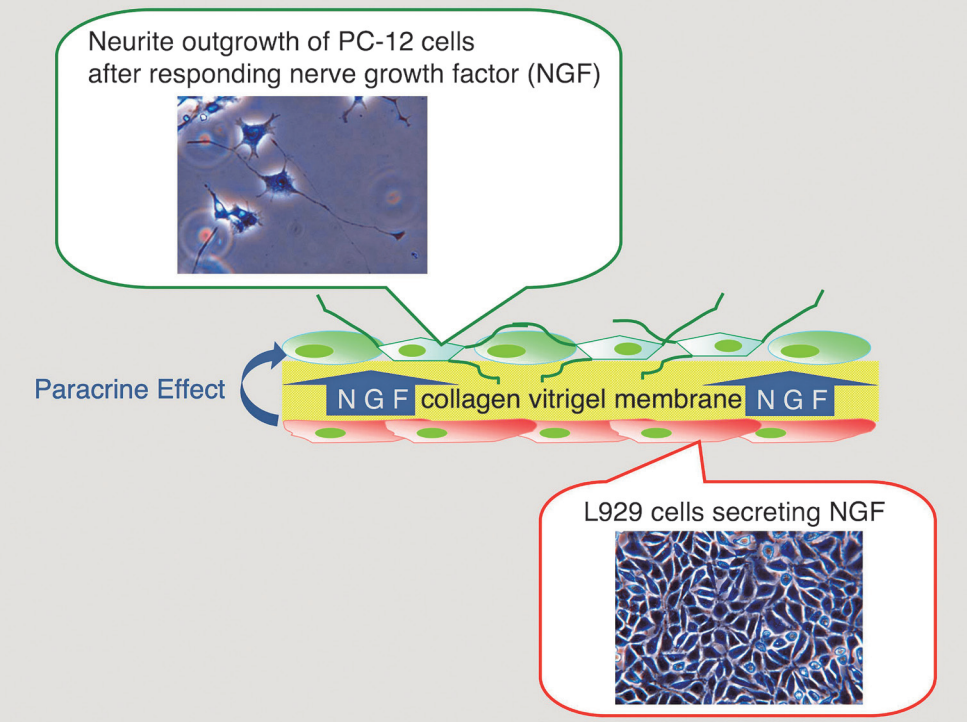

Fig. 3 Paracrine effect between different types of cells via a collagen vitrigel membrane scaffold

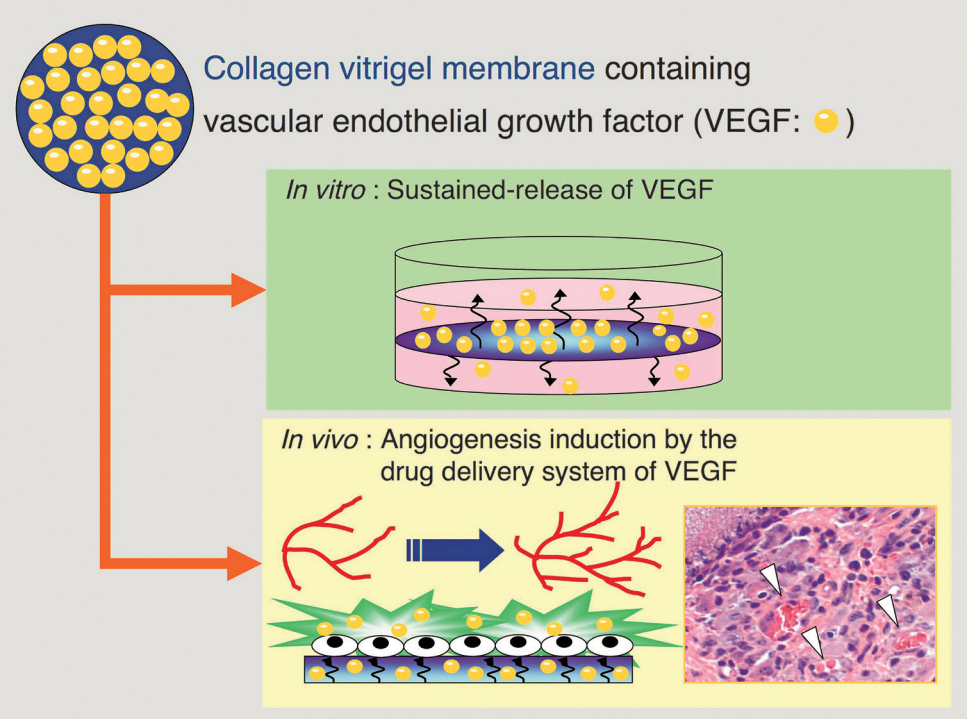

Fig. 4 Drug delivery system utilizing a collagen vitrigel membrane scaffold