immunohistostaining on day 55.

1) culture medium of CnT-30 medium (CELLnTEC) added with 1 nM BMP4

2) culture medium of defined K-SFM medium (Gibco/Invitrogen) added with 10% FBS and 1 nM BMP4

[0100]

(Results)

In a culture using the culture medium of any of the above-mentioned 1) and 2), the epithelial precursor tissue self-organized in the surface layer of the human ES cell aggregate permitted expansion culture and, on 55 days after the start of the differentiation culture, formation of an epithelial structure expressing cytokeratin 3 (CK3), cytokeratin 12 (CK12), cytokeratin 14 (CK14), p63, and ZO-1, which are specific to corneal epithelium, was observed in addition to being positive to Pan-cytoekeratin, in not less than 80% of the aggregates (Fig. 2). The results have clearly demonstrated that the epithelial tissue in the surface layer, which was sterically formed by the self-organization by the method of the present invention, is a precursor tissue of the cornea.

[0101]

Example 3: Self-organization of corneal precursor tissue having both epithelial tissue and mesenchymal tissue from human ES cells

(Method)

In the same manner as in Example 2, culture was performed up to day 30. That is, human ES cell aggregates were cultured in a V bottom 96 well plate under culture conditions of Example 1 up to day 18 of differentiation induction, then suspended aggregates were transferred to a cell nonadsorptive petri dish (diameter 6 cm), and suspension culture was performed at 37°C in the presence of 5% CO_2, 40% O_2. The culture medium used for the culture was gfCDM + 5% KSR added with 1 nM BMP4 from days 18 to 30, followed by analysis by immunohistostaining. A part

Human ES cell aggregates were cultured in a V bottom 96 well plate under the same conditions as in the above-mentioned Examples up to day 30 of differentiation induction. Thereafter, suspended aggregates were transferred to a cell nonadsorptive petri dish (diameter 6 cm), and suspension culture was performed under the conditions of 37°C, 5% CO_2, 40% O_2. As the culture medium, gfCDM + 5% KSR was used up to day 30, and gfCDM + 20% KSR was used from day 30. From days 3 to 18, 5 nM BMP4 was added. On day 24, the BMP4 concentration was reduced to half (2.5 nM), and continuously added at 1 nM from day 30~~—ff~~. In addition, basic FGF was added to the medium at a concentration of 20 ng/ml from day 15 during culture. The suspended aggregates were analyzed by immunohistostaining on day 84.

[0106]

(Results)

An epithelial precursor tissue self-organized in the surface layer of the human ES cell aggregate was found, and on 84 days after the start of the differentiation culture, corneal epithelium positive to cytokeratin 3 (CK3) specific to corneal epithelium, cytokeratin 12 (CK12), cytokeratin 15 (CK15) which is a marker of corneal epithelium stem cell was found (Fig. 5A, 5B and 5C). In addition, Na-K ATPase expressed on the corneal epithelium was also stained (Fig. 5D). The epithelium had a layered structure of the epithelium, which is characteristic of mature cornea, wherein the surface layer is squamous epithelium, and the deep layer is cuboidal epithelium (Fig. 5B). The results suggest the steric corneal epithelial precursor tissue formed by self-organization by the method of the present invention spontaneously performs stratification when it is matured further, and shows tissue construction inherent to the corneal epithelium and protein expression, which are close to those in the living body. Also, a mesenchymal aggregate layer was found below the corneal epithelium, which clearly demonstrates that they are precursor tissues of the total