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(54) Title: TEMPORARY BIOLOGICALLY ACTIVE COVER FOR EXTENSIVE WOUND AREAS AND METHOD OF PREPARING THEREOF

(57) Abstract

Temporary biologically active cover for extensive wound areas which comprises a support based on sparingly crosslinked hydrophilic polymers with cultivated keratinocytes. As hydrophilic polymers there are used hydrophilic polymers selected from the group consisting of poly(2-hydroxyethyl methacrylate), copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and sodium methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and methacrylic acid containing up 1 % of crosslinking agent. These polymers are commonly designated as hydrogels. Method for preparing a temporary biologically active cover which consists in the direct cultivation of keratinocytes on a preincubated hydrogel support. The use of a cover according to the invention characterized in that the keratinocytes cultivated on the support are directly transferred to the skin defect so that the cover is put onto the surface of the defect by the side with the grown keratinocytes. The keratinocytes migrate from the surface of the support and colonize the surface of the skin defect. Due to that the isolation of the cells from the bottom of the cultivation vessel and their footing onto a transplantation support falls away and the transplantation of the cells is very simplified.

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Description

Temporary biologically active cover for extensive wound areas and method of preparing thereof

Technical field

The invention pertains to a temporary biologically active cover for extensive wound areas, the method of preparing thereof and the method of it's use.

Background Art

For covering extensive skin defects such as burns, calf-ulcers (ulcus cruris) are used cultivated autologous keratinocytes. This method is based on the excision of a small piece of skin (3 cm^2), from which the skin cells (keratinocytes) are isolated. These cells are grown under the conditions for tissue cultures, enzymatically released from the bottom of the cultivation vessel, in the form of a continuous growth footed on a vaseline gauze and subsequently transferred to the skin defects. Thus it is possible to prepare from relatively small skin pieces covers for extensive areas of the damaged skin (Green et al., Proc. Natl. Acad. Sci. USA, 76, 5665-5668, 1979). The problem remains in the transfer itself which is technically very complicated and can be the cause of the failure.

We have found that it is possible to cultivate the keratinocytes on a hydrogel support and to use it for the subsequent transfer onto the skin defect as temporary biologically active cover.

Disclosure of Invention

The object of the present invention is a temporary biologically active cover for extensive wound areas which comprises a support based on sparingly crosslinked hydrophilic polymers with cultivated keratinocytes. As hydrophilic polymers according to the invention there are used hydrophilic polymers selected from the group comprising poly(2-hydro-

xyethyl methacrylate), copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and sodium methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and methacrylic acid containing up 1% of crosslinking agent. These polymers are commonly designated as hydrogels.

Another object of the invention is a method for preparing the temporary biologically active cover which consists in the direct cultivation of keratinocytes on a preincubated hydrogel support.

The use of the cover according to the invention is characterized in that the keratinocytes cultivated on the support are directly transferred to the skin defect so that the cover is put onto the surface of the defect by the side with the grown keratinocytes. The keratinocytes then migrate from the surface of the support and colonize the surface of the skin defect.

Due to that the isolation of the cells from the bottom of the cultivation vessel and there footing onto a transplantation support falls away and the transplantation of the cells is very simplified.

The procedure takes place in such a way that from a patient there is taken a small dermoepidermal graft having a thickness of about 0.2-0.3 mm from which the keratinocytes are released by the treatment with a trypsine solution. To the bottom of the cultivation vessel is placed a hydrogel support in disk form being 24 to 48 hours preincubated with a 10 to 25% bovine serum. On bottom of the cultivation vessel with the hydrogel support are seeded lethally irradiated (6000 rad) 3-T-3 mouse fibroblasts (feeding cells) in a density of $1,3 \times 10^6$ per 50 cm^2 promoting the adherence and the first phase of growth of keratinocytes (Green et al., 1979). After the adherence of the fibroblasts there were seeded the keratinocytes in a density of 2×10^6 per 50 cm^2. The cells are cultivated at 37°C in the atmosphere of 3.3% CO_2.

There is used the cultivation medium containing commonly

1) Eagl MEM with unessential aminoacids and sodium pyruvate (Institute of Molecular Genetics of ASC, Prague), 2) 0.3 mg/ml Glutamine (ÚSOL, Prague), 3) 10% Bovine serum (Bioveta, Opava), 4) Hydrocortisone (0.5 µm/ml) (Hydrocortisone Spofa soluble, 5) Peniciline (200 units/ml), Streptomycine (10^{-4}g/ml), 6) Insulin (5 µg/ml) (Actrapid MC NOVO, Denmark), 7) Choleratoxin (10^{-10} M) (Sigma), 8) 10 ng/ml Epidermal growth factor (Sigma).

The invention is illustrated by the following Examples.

Examples

Example 1

To the bottom of the cultivation vessel a support having the form of a swollen hydrogel disk or net with the diameter of 25 mmm prepared from the copolymer 2-hydroxyethyl methacrylate (70 wt.%) with diethylglycol methacrylate (30 wt.%) crosslinked with ethylene dimethacrylate (0.3 wt.%) containing 47% of water. The swollen disk was 24 hours preincubated with 25% (v/v) bovine serum. On the bottom of the cultivation vessel with the hydrogel support were seeded lethally irradiated (6000 rad) 3-T-3 mouse fibroblasts (feeding cells) promoting the adherence and the first phase of growth of keratinocytes in a density of 1,3 x 10^6 per 50 cm^2. After the adherence of the fibroblasts there were seeded the keratinocytes in a density of 2 x 10^6 per 50 cm^2. The cells are cultivated at 37°C in the atmosphere of 3.3% CO_2.

The keratinocytes for the cultivation were obtained by taking from the patient a fine dermoepidermal graft having the thickness of about 0.2-0.3 mm, from which the keratinocytes have been released by the treatment with a trypsine solution.

The used cultivation medium consisted of the 1) Eagl MEM with unessential aminoacids and sodium pyruvate (Institute of Molecular Genetics of ASC, Prague), 2) 0.3 mg/ml Glutamine (ÚSOL, Prague), 3) 10% Bovine serum (Bioveta, Opava), 4) 0.5 µm/ml Hydrocortisone (Hydrocortisone Spofa soluble, 5) Peniciline 200 units/ml, Streptomycine 10^{-4}g/ml, 6) 5 µg/ml

Insulin (Actrapid MC NOVO, Denmark), 7) 10^{-10} M Choleratoxin (Sigma), 8) 10 ng/ml Epidermal growth factor (Sigma).

Example 2

There was used the procedure as in Example 1 with the difference, that as support in form of a swollen disk there was used the polymer poly(2-hydroxyethyl methacrylate) (polyHEMA) crosslinked with 1 wt. % of ethylene dimethacrylate.

Example 3

The same procedure as in Example 1 was carried out with the difference that as support there was used a swollen disk made from the copolymer 2-hydroxyethyl methacrylate (HEMA) (80 wt.%), diethylglycol methacrylate (DEGMA) (20 wt.%) and methacrylic acid (0.5 wt.%) crosslinked with ethylene dimethacrylate (0.5 wt.%).

Example 4

There was used the same procedure as in Example 1 differing in that as support there was used the copolymer 2-hydroxyethyl methacrylate (HEMA) (70 wt. %), diethylglycol methacrylate (DEGMA) (30 wt.%) and methacrylic acid (0.5 wt.%) crosslinked with ethylene dimethacrylate (0.3 wt.%).

CLAIMS

1. Temporary biologically active cover for extensive wound areas characterized in that it consists of a support based on sparingly crosslinked hydrophilic polymers with cultivated keratinocytes.

2. Temporary biologically active cover for extensive wound areas according to claim 1 characterized in that as hydrophilic polymers there are used hydrophilic polymers selected from the group comprising poly(2-hydroxyethyl methacrylate), copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and sodium methacrylate, copolymer of 2-hydroxyethyl methacrylate with diethylglycol methacrylate and methacrylic acid containing up 1% of crosslinking agent.

3. Method for preparation of a temporary biologically active cover according to claim 1 characterized in that the keratinocytes are directly cultivated on the preincubated hydrogel support.

4. Method for preparation of a temporary biologically active cover according to claim 2 characterized in that the keratinocytes are directly cultivated on the preincubated hydrogel support.

5. Use of a temporary biologically active cover for extensive wound areas according to claim 1 characterized in that the cultivated keratinocytes are transferred to the skin by putting the cover onto the surface of the defect by the side grown with keratinocytes.

6. Use of a temporary biologically active cover for extensive wound areas according to claim 2 characterized in that the cultivated keratinocytes are transferred to the skin by putting the cover onto the surface of the defect by the side grown with keratinocytes.

INTERNATIONAL SEARCH REPORT

Int ional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,88 08448 (BAY MICHAEL) 3 November 1988 see page 10, line 10 - line 29 see page 11, line 3 - line 20 ---	1-6
A	WO,A,90 00595 (BANES ALBERT J) 25 January 1990 see claims ---	1-6
A	DATABASE WPI Section Ch, Week 9208 Derwent Publications Ltd., London, GB; Class A96, AN 92-060489 & JP,A,04 004 868 (TORAY IND INC) , 9 January 1992 see abstract --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRITISH JOURNAL OF PLASTIC SURGERY, vol. 46, no. 3, 1993 GB, pages 228-234, N. CARVER ET AL. 'THE EFFECT OF BACKING MATERIALS ON KERATINOCYTE AUTOGRAFT TAKE' see page 229 ---	1
P,X	CELL BIOLOGY INTERNATIONAL, vol. 18, no. 5, 1994 page 558 B. DVORANKOVA ET AL. 'INTERACTION OF KERATINOCYTES WITH HYDROGELS' see page 558 -----	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/CZ 95/00011

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WO-A-8808448	03-11-88	AU-B-	614747	12-09-91
		AU-B-	1685288	02-12-88
		EP-A-	0355112	28-02-90
		JP-T-	2504221	06-12-90
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