

OPTIMIZATION AND STORAGE OF MICRO-FABRICATED SKIN SUBSTITUTES

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ABSTRACT

Several variations of skin equivalents are under development or are presently commercially available and there has been some clinical success for their use in the treatment of the burned patient. However, none of the skin equivalents are true replicas of native skin so their ability to restore normal skin function is limited [1,2,3]. The goal of the current study is to create a tissue scaffold that must act as a suitable carrier for cell culture and to dry cells on these special patterns to low moisture content for a long-term storage. As a first step we optimized the geometry of polydimethylsiloxane (PDMS) surface where we were able to culture cells that grew uniformly. Next we desiccated KGF secreting fibroblasts on these surfaces to moisture content as low as 5% and showed that the KGF secretion post rehydration is similar to control cells. This is a first attempt to supply a true dermal environment and a basal lamina analogue with special pattern would allow for media diffusion while serving as a point of attachment for cultured epithelial cells and a separation barrier from underlying fibroblasts.

INTRODUCTION

At the very beginning, we tried to find a special material so that we can culture cells on the surface. Up to now we had successfully cultured cells on natural material like pure cotton, Biobrane and our own design pattern of polydimethylsiloxane silicone elastomer. Recently, microfabrication technologies, originally developed by the computer industry to make integrated circuits, have found novel application in biology and medicine where there is a need to create small complex structures with micron scale dimensions.

MATERIALS AND METHODS

Micro fabrication and PDMS negative replicates

A soft lithography approach was used to produce silicon wafer with some surface features that were designed by AutoCAD and then created by photolithography of silicon wafers spin coated with SU-8 polymer. Negative replicates of these silicon wafers were produced using a polydimethylsiloxane silicone elastomer (PDMS).

NIH/3T3 fibroblasts culture medium and dry process

Culture medium was changed every 3-4 days with 90% Dulbecco's Modified eagles Medium (DMEM), 10% Bovine Calf serum (BCS) and 1% Penicillin-Streptomycin (P/S) [4]. Trypsinized cells by standard protocol and spin cells down and resuspend cells in 0.2 M trehalose in 1/3 RPMI Determined cell concentration using a Hemocytometer and adjust volume of cell suspension to yield desired cells/ml. Placed droplets in each plate and determine wet of each sample and placed the dishes to be dried in a Vacuum dryer, after that dry samples to desired percent of residual moisture content at ambient temperature. Vacuumed pressure is recommended 29.5" HG, also placed the undried control plates into a place with 100% relative humidity for the identical time as the dried sample. Weight each dish after dry, then rehydrated with desired volume of pre-warmed culture medium immediately. Gently scraped the surface of the culture dish to ensure the cells have detached, and then placed dishes in 37 °C incubator over night.

Here, NIH/3T3 fibroblasts were genetically modified to express keratinocyte growth factor (KGF) [5,6] by retroviral transduction. These KGF secreting cells were dehydrated by convective drying methods to 20, 10, and 5% relative moisture content. After drying cells were immediately rehydrated with warm culture medium. At defined intervals culture medium was harvested and a total cell count was obtained. KGF levels in the medium were assayed by sandwich ELISA and normalized to cell number.

RESULTS AND DISCUSSION

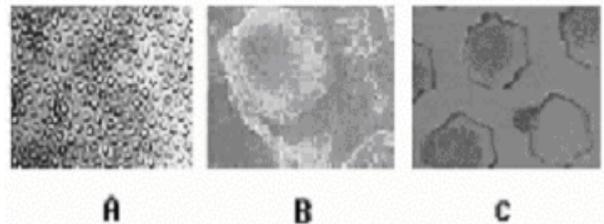


Figure 1: Cells grow on different material surface. A, B and C patterns are hexagons. A is r25s25; B is r300s300; C is r150s150 (r is radii in μm and s is space in μm).

Different surface geometries were created using soft lithography as described previously. As shown in Fig. 1, cells tend to clump after 24 hours in the r150s150 and r300s300 patterns. Cells would spread very well in r25s25 pattern. Because these very small patterns (r25s25) destroy the surface tension, so we can culture cells on it. We also used other surface geometries like channels at different widths and found that none of them were suitable for cell culture.

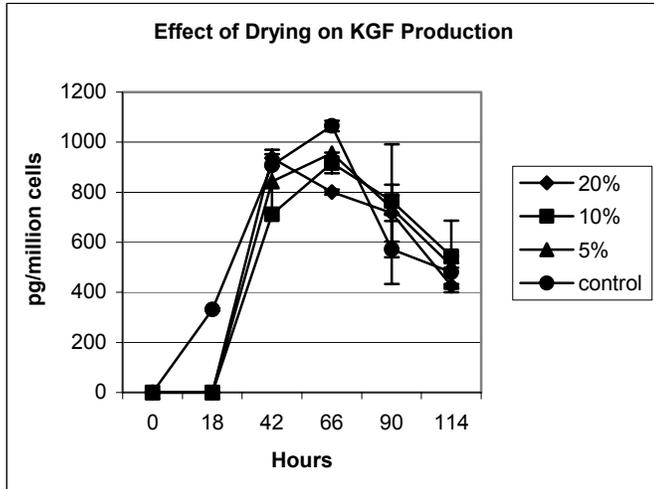


Figure 2: Effect of drying on KGF production. % Moisture = (Dry weight –baked weight)/(wet weight-baked weight) * 100.

Undried control cells had steady rate of KGF production for the first 48 hours and reached a maximum expression of 1060pg/million cells at 66 hours post drying. Each of the dried conditions showed a similar expression profile when compared to undried control with two (2) differences. First, there was a delay in KGF production post drying. This may be caused by a recovery phase the cells undergo in response to a dehydrated state. The second difference between controls and dehydrated cells was the initial rate of production. After the first 18 hours the rate of production for controls was 24pg/hour for 24 hours. In contrast the initial rate of production for 20, 10, and 5% relative moisture after the first 18 hours was 39, 30 and 35pg/hour respectively for 24 hours. The reasons for this delay in KGF production and increase in the initial rate of production are not understood but may have therapeutic advantages when used in a biological dressing.

In summary our ultimate goal is to make three layer textile products with top layer permeable, bottom layer impermeable and the core layer created with dried cells for incorporation into a textile product or special PDMS pattern. We've used a microfabrication process based on photolithography and PDMS replica to make analogs of the basement membrane with complex topographic features. The photolithography process significantly improves the precision with which we can control the micro-scale surface topography of these membranes. We also cultured cells on this it and dried to desired moisture content and were able to get function.

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