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Supporting Information

Three-dimensional hierarchical cultivation of human skin cells on bio-adaptive hybrid fibers

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Materials and Methods

Cell counting

For cell counting, each individual PCL, blend and hybrid fiber mat was transferred to a new well in order to prevent trypsination of cells attached to the well bottom. Removal of medium residuals and cell debris from the fiber mats was performed by washing the samples with PBS for three times. The subsequent extraction of the cells was carried out by enzymatic digestion using trypsin for eight minutes at 37°C. Gently tapping of the well plates facilitated the subsequent cell extraction from the fiber mats and increased the cell yield. To stop the trypsin effect, medium supplemented with fetal calf serum was added to the wells. Pipetting of the medium/trypsin mixture up and down for several times further promoted cell detachment. After collecting the medium/trypsin mixture, each fiber mat was additionally rinsed with fresh medium to allow for harvesting all detached cells still remaining on the fiber mat surface and thus contributing to the cell counts. The cell suspension was finally analysed based on the non-invasive electrical current exclusion (ECE) principle using CASY® Cell Counter Model TT (Roche Innovatis AG, Bielefeld, Germany).

Results and Discussion

To verify our findings regarding the supportive effect of hybrid fibers on cell proliferation based on qualitative analysis using fluorescence imaging in comparison to pure PCL and blend fibers, we further addressed this important point by additional quantitative cell counting experiments. By detaching the cells from the scaffold matrix after different time point of cultivation (from day 1 to day

14) using enzymatic digestion (trypsin), we found considerable differences in terms of cell numbers among the three types of fiber mats (Figure S1 A). Therefore, these results demonstrate and confirm our previous findings regarding the positive effect of the bio-adaptive hybrid fibers on cell migration and proliferation and allow for an absolute estimation of cell counts. However, these data even underestimate this effect, as unfortunately not all cells could be completely be detached from the fibers and thus are not contributing to the counts. This effect is obviously more pronounced for fibers, which provide optimal cell attachment and proliferation as particularly concerning the hybrid fibers. For illustration, we performed counterstainings of the different fiber types after cell detachment (Figure S1 B). While all cells from the PCL fibers are detached, blend and (even more) hybrid fibers still contain cells. We evaluated different incubation intervals for enzymatic digestion to extract the cells from the fibers, however, unfortunately further prolongation of incubation time let to lysis of already detached cells. Taken this into consideration, the positive effect of the hybrid fibers on cell behaviour as shown for Figure S1 A is even significantly underestimated.

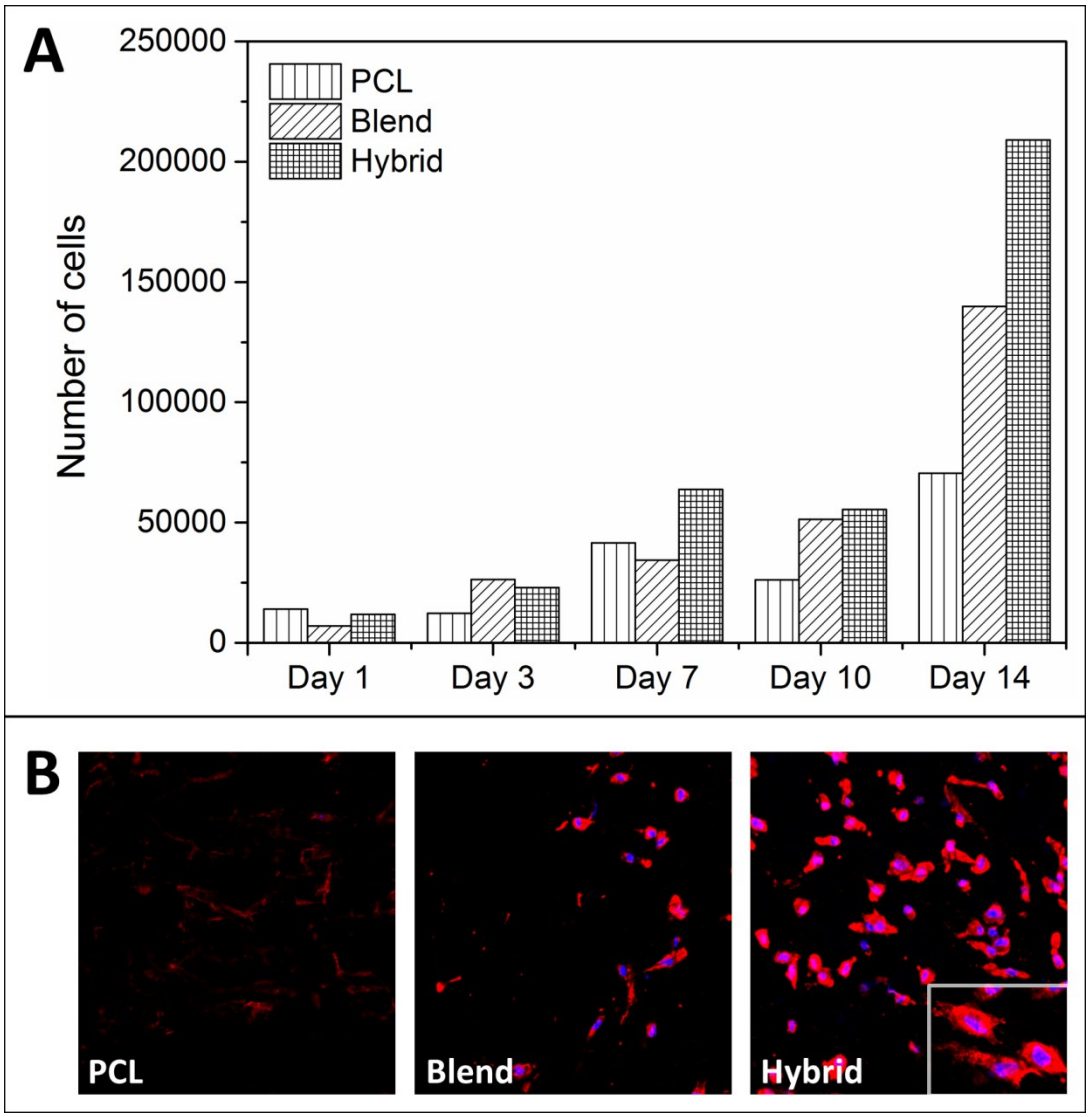

Fig. S1. Quantitative evaluation of the proliferation capacity of human dermal fibroblasts cultivated on PCL, blend and hybrid fibers. (A) Determination of cell numbers by detaching the cells from the fibers using enzymatic digestion (trypsin) at specific time points within a cultivation period of 14 days. (B) Counterstaining of PCL, blend and hybrid fibers after the cell detachment process to verify complete cell extraction using fluorescence staining for visualization of the cell membrane (red) and cell nuclei (blue).