a regenerative niche. Cell-cell and cell-matrix interactions remain a central element of this activity. One of the paradigm shifts we need to master is the step from what is usual even in complex cell biological models, namely the use of purely physiological conditions, to a more realistic situation as would be found in the clinical setting. Thus, we need to understand regeneration in hostile environments, which include post-trauma, cancer and multimorbidity. This will be discussed with examples from the author's own research.

One of the important in vitro methods to investigate the mechanisms involved in regeneration is the use of coculture systems with relevant human cells, usually on tissue culture plastic and, as knowledge progresses, on more complex 3D biomaterial scaffolds. As major limiting factors in bone regeneration are the speed and extent of vascularization, we have established human osteoblast (pOB)-endothelial cell (EC) cocultures to study cellular crosstalk and its possible use for translational strategies [1,2].

Concerning the background, if two cell populations, that is, human pOB and human dermal microvascular EC (HDMEC), are seeded as cell suspensions on an open porous biomaterial scaffold, such as can be made from microfibres of the silk protein fibroin, the two cell types will interact in such a way that lumen-containing, capillary-like structures (CLS) will form as a vascular network [3]. Further molecular studies on the cellular crosstalk revealed that the EC induce an upregulation of growth factor and matrix production in pOB, such as VEGF and collagen type I resp. The EC then respond to these signals by promoting the angiogenic phenotype [4,5].

The following additional approaches have been adopted to study CLS formation: use of early embryonic signals, such as sonic hedgehog (shh), to accelerate both osteo- and angiogenesis [6,7], use of intermittent hypoxia, but not constant hypoxia, to promote vascular sprout formation, and study of possible stimulatory roles for macrophages in the bone regenerative niche [8]. How this is investigated in coculture models will be discussed in the context of future developments. Naturally, all phenomena from in vitro studies require proof of concept in relevant in vivo models, as only this approach can lead to a translational perspective. Thus, we were able to demonstrate that these in vitro pre-formed vessels can rapidly become inosculated, that is, incorporated into the pre-existing microcirculation of host tissue in a subcutaneous implantation model [9]. The major role of the osteoblasts as a natural „drug delivery system" was shown by the fact that host vascular response can be stimulated by these cells even in the absence of a pre-cultivation with endothelial cells [10].

A further aspect offering a promising perspective for the future is NanoMedicine, which uses advances in nanotechnology for medical applications. For reasons of time this will not be addressed in the context of the presentation.

In conclusion, biomaterials, especially so-called responsive biomaterials, are an essential element of modern regenerative medicine, and must be accompanied by state of the art life sciences, from cell and molecular biology to good clinical practice. To achieve this the multidisciplinary approach is a conditio sine qua non.

[Engineering of Biomaterials, 128-129, (2014), 1-2]

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NANOFIBROUS MEMBRANE WITH FIBRIN AND COLLAGEN STRUCTURES AS CARRIERS FOR SKIN CELLS

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[Engineering of Biomaterials, 128-129, (2014), 2-4]

Introduction

Recently, nanofibrous materials have been interesting for applications in tissue engineering. They better simulate the structure of fibrous component of natural extracellular matrix than conventional flat or microstructured surfaces and they enable adsorption of cell adhesion mediating molecules in an appropriate spatial conformation. The appropriate spatial conformation enables a good accessibility of active sites on these molecules by adhesion receptors on the cell membrane [1,2]. Most of the clinically used skin substitutes consist of non-resorbable material and allogeneic cells thus they cannot provide permanent coverage due to their final rejection. The promising approach could be construction of nanofibrous carriers from biodegradable polymers which will be slowly resorbed in organism and finally replaced by regenerated tissue. The attractiveness of the nanofibrous membrane for adhesion and growth of skin cells can be further promoted by coating the membrane with biomolecules normally presented in natural skin (collagen, hyaluronan) or occurring during wound healing (fibrin).

The study is focused on evaluation of adhesion and growth of human dermal fibroblasts and human immortal HaCaT keratinocytes on polylactide (PLA) nanofibrous membranes coated with fibrin, collagen and fibronectin.

Materials and methods

The PLA membranes were prepared using the novel Nanospider needleless electrospinning technology. We coated the membranes with fibrin, fibrin with fibronectin (cell adhesion-mediating extracellular matrix protein), collagen I or collagen I with fibronectin.

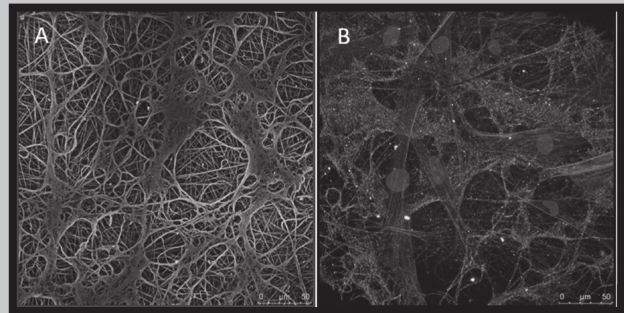

FIG. 1. Immunofluorescence staining of fibrin structure on PLA membranes (A) and phalloidin staining of F-actin (red) and nucleus staining (Hoechst 33342, blue) in human dermal fibroblasts after 24 hours of cultivation on PLA membranes with immunofluorescence stained fibrin structure (green) (B). Leica TCS SPE DM2500 confocal microscope, obj. 40 oil, bar 50 μm.

We evaluated adhesion, morphology, proliferation, metabolic activity (determined by MTS assay) and viability (determined by Live/dead assay) of dermal human fibroblasts and human immortal HaCaT keratinocytes. We also studied collagen production (real-time PCR, immunofluorescence staining) by fibroblasts stimulated by fibrin structure on PLA nanofibrous membrane.

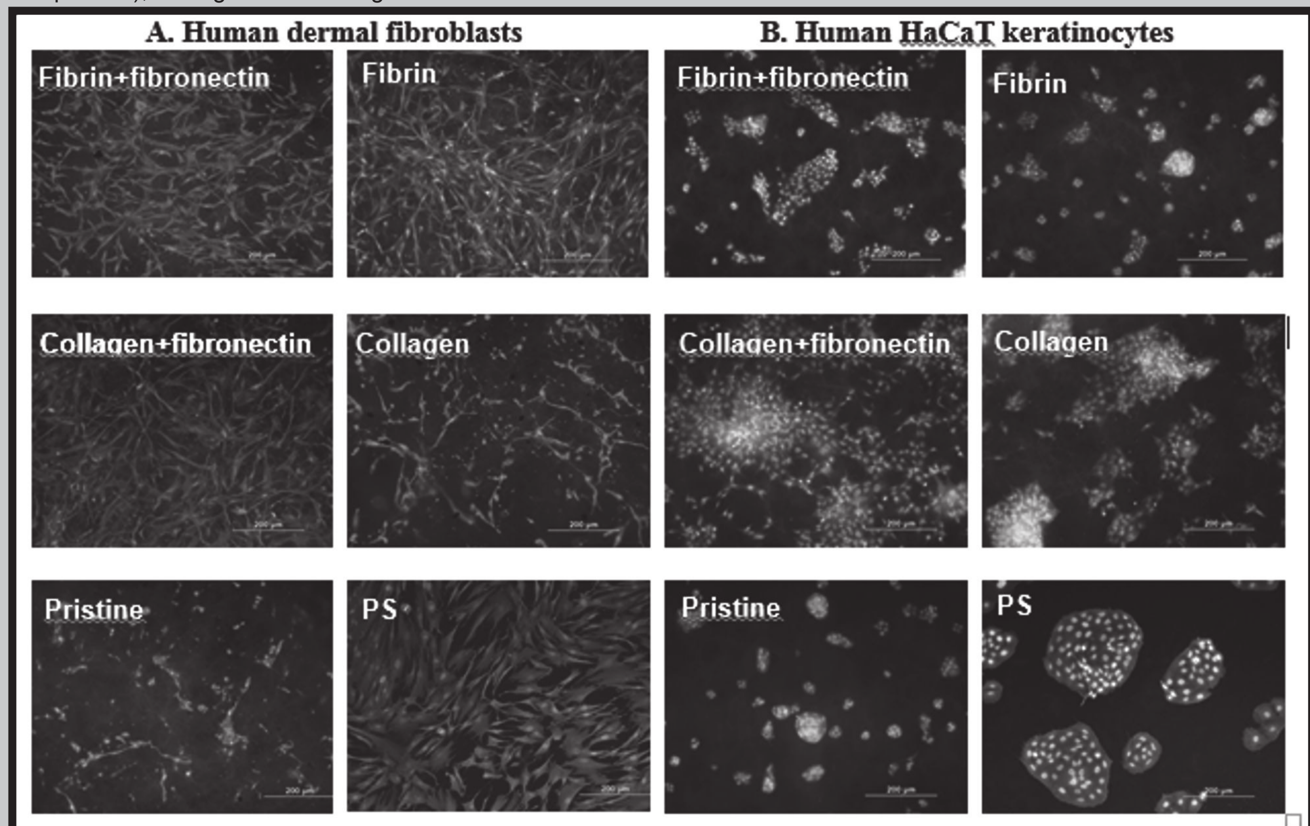

FIG. 2. Morphology of human dermal fibroblasts (A) and human HaCaT keratinocytes (B) after 3 day-cultivation on pristine PLA membranes, PLA membranes with fibrin and fibronectin, fibrin, collagen and fibronectin or collagen. Standard cell culture polystyrene dish (PS) served as a reference material. Cells stained with Texas Red C2-Maleimide and Hoechst #33342. Olympus IX 51 microscope, obj. 10 x, DP 70 digital camera, bar 200 μm.

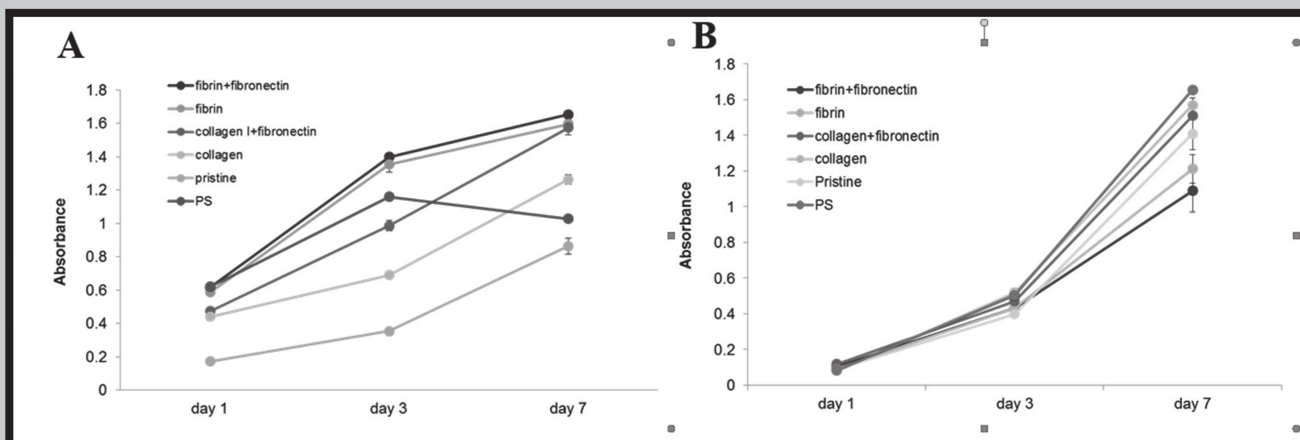

FIG. 3. Mitochondrial activity of human dermal fibroblasts (A) and human HaCaT keratinocytes (B) determined by MTS assay on day 1, 3 and 7 after cell seeding on pristine PLA membranes, PLA membranes with fibrin and fibronectin, fibrin, collagen and fibronectin or collagen. Standard cell culture polystyrene dish (PS) served as a reference material. Arithmetic means ± S.E.M from 9 measurements made on three independent samples for each experimental group and time interval.

Results

Results indicate that PLA nanofibrous membrane promoted adhesion and growth of the skin cells. Fibrin (FIG.1) and collagen structures on PLA membranes further improved adhesion, proliferation and metabolic mitochondrial activity of the skin cells. The human dermal fibroblasts preferentially adhered and were more spread on the membranes coatedwith fibrin, fibrin with attached fibronectin on its surface or collagen I with fibronectin than on the membranes coated only with collagen or on the membranes in pristine form (FIG.2A). Moreover, the metabolic activity of human dermal fibroblasts was the highest on the membranes coated with fibrin or fibrin with fibronectin (FIG.3A). In addition, fibrin structures on PLA membranes stimulate fibroblasts to produce collagen I. The membranes coated with collagen I or collagen I with fibronectin promoted spreading of the HaCaT keratinocytes and increased the cell metabolic activity in comparison with pristine membranes or membranes coated with fibrin or fibrin with fibronectin (FIG.2B, 3B). Viability (determined by a Live/Dead assay) of the fibroblasts and the keratinocytes on the membranes was almost 100% on all samples.

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BLACK ORLON AS PROMISING MATERIAL FOR BONE TISSUE ENEGINEERING

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[Engineering of Biomaterials, 128-129, (2014), 4-6]

Introduction

Black Orlon is a promising material for applications in tissue engineering and regenerative medicine. Chemically, it is a carbonized polyacrylonitrile (PAN) containing fibrous ladder structure with chemical functional groups containing oxygen (e.g., hydroxyl, carboxyl), created by heating of Orlon above 300° C in the air atmosphere [1]. Although the biomedical applications of this materials started relatively early (in the seventieth), its potential for these applications has not yet been fully explored. In 1976, black Orlon was tested for construction of blood-contacting anticoagulant surfaces in the form of atrial patches implanted into hearts of experimental dogs [2].

Black Orlon can be relatively easily processed into three-dimensional (3D) scaffolds with microporous structure [3], and also nanofibrous structure created by electrospinning [4]. Microporous 3D scaffolds of carbonized PAN showed excellent osteoinductivity, i.e., they promoted osteogenic differentiation of human bone marrow mesenchymal stem cells without addition of other osteogenic factors [3]. The nanofibrous structure is also of a great importance for bone tissue engineering. It has been reported that nanos-