A full-thickness skin construct made of a collagen hydrogel strengthened by a fibrin-modified nanofibrous membrane

Julia Pajorova[1,2], Marketa Bacakova[1], Antonin Broz[1], Martina Travnickova[1,2], Lucie Bacakova[1]

[1]*Dept. of Biomaterials and Tissue Engineering, Institute of Physiology CAS, Prague, Czech Republic*
[2]*Second Faculty of Medicine, Charles University, Prague, Czech Republic*
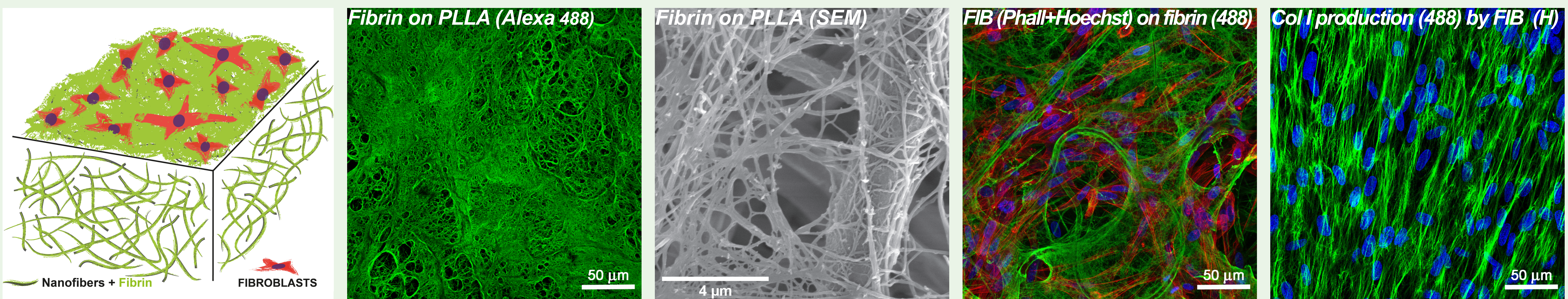
Introduction

For many years, a three-dimensional (3D) collagen hydrogel has been used for embedding the cells to provide them with physiological 3D environment. However, the stiffness of tissue-engineered skin analogues based on collagen hydrogels is insufficient. The cell traction forces typically cause the shrinkage of the hydrogels. In order to improve the mechanical properties of a two-layer skin construct, we stabilized the collagen hydrogel by fibrin-modified nanofibrous membrane. In order to pre-vascularize the skin model, we co-cultured the endothelial cells with adipose-derived stem cells.
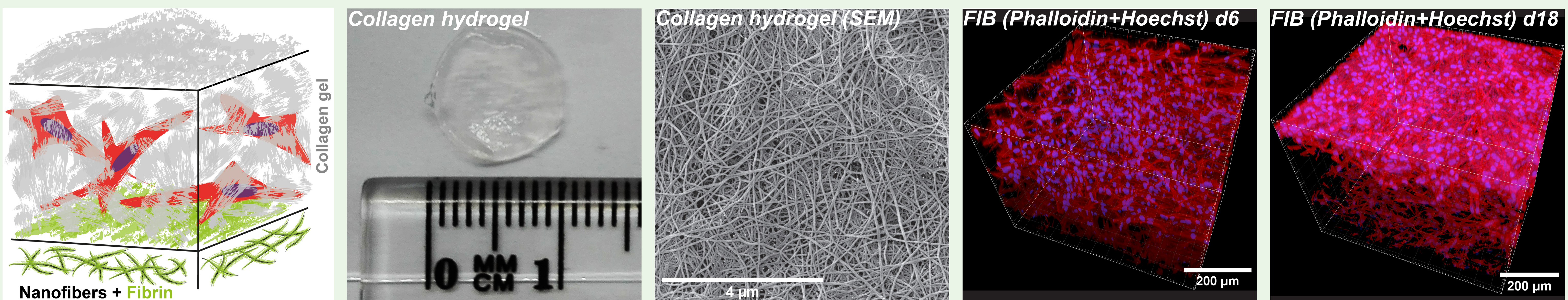
Materials & Methods

Nanofibrous polylactid acid (PLLA) membranes were modified with fibrin homogenous mesh. Fibrin-modified membranes were seeded either with human dermal fibroblasts (FIB) or with adipose-derived stem cells (ASC). After 3 days, collagen hydrogel was applied either with endothelial cells (HUVEC) or without them on the surface of fibrin-modified membranes pre-seeded either with fibroblasts or with stem cells. The primary human keratinocytes (KERAT) were seeded on the surface of collagen hydrogel. Cell proliferation, migration and morphology were evaluated by high-resolution microscopy.
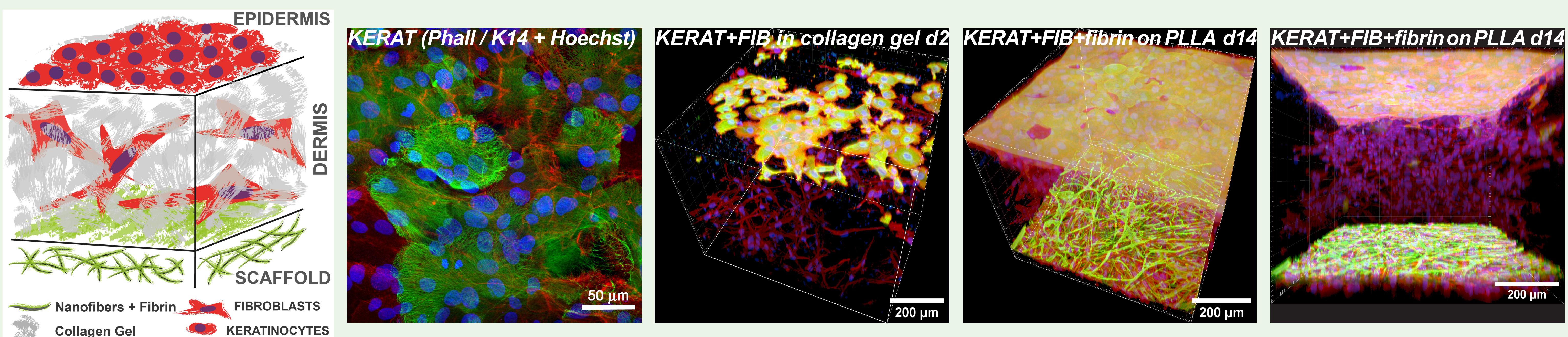
Results

1. Fibroblasts on fibrin-coated PLLA membrane

Nanofibers + Fibrin — FIBROBLASTS

Fibrin on PLLA (Alexa 488) — 50 µm
Fibrin on PLLA (SEM) — 4 µm
FIB (Phall+Hoechst) on fibrin (488) — 50 µm
Col I production (488) by FIB (H) — 50 µm

2. Fibroblasts migrated into collagen hydrogel from fibrin-modified PLLA membrane

Collagen gel — Nanofibers + Fibrin

Collagen hydrogel
Collagen hydrogel (SEM) — 4 µm
FIB (Phalloidin+Hoechst) d6 — 200 µm
FIB (Phalloidin+Hoechst) d18 — 200 µm

3. Bilayered construct of fibroblasts and keratinocytes strengthened by fibrin-modified membrane

EPIDERMIS — DERMIS — SCAFFOLD
Nanofibers + Fibrin — FIBROBLASTS
Collagen Gel — KERATINOCYTES

KERAT (Phall / K14 + Hoechst) — 50 µm
KERAT+FIB in collagen gel d2 — 200 µm
KERAT+FIB+fibrin on PLLA d14 — 200 µm
KERAT+FIB+fibrin on PLLA d14 — 200 µm

4. Pre-vascularization of bilayered construct by HUVEC and ASC (Current work)

Collagen gel — Nanofibers + Fibrin
ASC — HUVEC

HUVEC on plastic d7 — 100 µm
HUVEC (Cell trecker) d14
HUVEC+ASC (Phall+Dapi) d14
HUVEC+ASC in collagen gel