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RESEARCH ARTICLE

Abnormalities in reparative function of lung-derived mesenchymal stromal cells in emphysema

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Abstract

Mesenchymal stromal cells (MSCs) may provide crucial support in the regeneration of destructed alveolar tissue (emphysema) in chronic obstructive pulmonary disease (COPD). We hypothesized that lung-derived MSCs (LMSCs) from patients with emphysema are hampered in their repair capacity, either intrinsically or due to their interaction with the damaged microenvironment. LMSCs were isolated from the lung tissue of controls and patients with severe emphysema and characterized at baseline. In addition, LMSCs were seeded onto control and emphysematous decellularized lung tissue scaffolds and assessed for deposition of extracellular matrix (ECM). We observed no differences in surface markers, differentiation/proliferation potential, and expression of ECM genes between control- and COPD-derived LMSCs. Notably, COPD-derived LMSCs displayed lower expression of *FGF10* and *HGF* messenger RNA (mRNA) and hepatocyte growth factor (HGF) and decorin protein. When seeded on control decellularized lung tissue scaffolds, control- and COPD-derived LMSCs showed no differences in engraftment, proliferation, or survival within 2 wk, with similar ability to deposit new matrix on the scaffolds. Moreover, LMSC numbers and the ability to deposit new matrix were not compromised on emphysematous scaffolds. Collectively, our data show that LMSCs from patients with COPD compared with controls show less expression of *FGF10* mRNA, HGF mRNA and protein, and decorin protein, whereas other features including the mRNA expression of various ECM molecules are unaffected. Furthermore, COPD-derived LMSCs are capable of engraftment, proliferation, and functioning on native lung tissue scaffolds. The damaged, emphysematous microenvironment as such does not hamper the potential of LMSCs. Thus, specific intrinsic deficiencies in growth factor production by diseased LMSCs may contribute to impaired alveolar repair in emphysema.

emphysema; extracellular matrix; growth factors; lung tissue repair; MSCs

INTRODUCTION

Emphysema is one of the hallmarks of chronic obstructive pulmonary disease (COPD), which according to the WHO is the third leading cause of death (1). The disease is characterized by irreversible loss of alveolar tissue and destruction of the extracellular matrix (ECM) microenvironment. The main risk factor for COPD is the inhalation of noxious gases, including cigarette smoke, air pollution, and job-related exposures, leading to oxidative stress, damage, inflammation, and abnormal tissue repair responses in susceptible individuals. Impaired tissue repair in emphysema may be due to exhaustion of stem and progenitor cell function (2) and dysfunction of supportive cell populations due to genetic defects in combination with changes in the microenvironment. In emphysema, destruction of lung tissue occurs, with altered presence of various ECM components, such as elastin, fibrillin-1, dermatan sulfate decorin, and heparan sulfate proteoglycans (3–6).

Mesenchymal stromal cells (MSCs) are a supportive cell type that can be found in multiple tissues of the adult body, including bone marrow, adipose tissue, and lung (7). MSCs have received much attention for their regenerative capacity (8), not only contributing to tissue renewal, but also, through paracrine effects, supporting site-specific epithelial and endothelial responses and suppressing inflammatory responses (9). However, MSCs from patients with emphysema may be limited in their reparative function, possibly due to long-term oxidative stress exposure and aging. For example, Bustos et al. (10) showed that bone-marrow MSCs (BM-MSCs) isolated from older mice have reduced anti-inflammatory properties compared with younger animals. Similarly, Denu et al. (11) highlighted the negative impact of a high-level

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reactive oxygen species (ROS) environment, reducing MSC proliferation, differentiation, and self-renewal.

Potentially, the damaged lung ECM-microenvironment contributes to disturbed tissue repair responses by impairing the function of supportive stromal cells, for instance, as proteoglycans capture and release growth factors to these cells. Furthermore, MSCs require specific ECM components, such as fibronectin, for their migration to sites of damage, survival, and function (9), which may be impaired in emphysema. In addition, degradation of ECM molecules by proteolytic enzymes that are increased in COPD may result in generation of small ECM fragments that can activate intracellular signaling of MSCs via toll-like receptors (12). Dependent on the activated receptor, this can induce either a proinflammatory or repair-orientated phenotype (12–14).

Intrinsic genetic defects and/or smoking-induced epigenetic defects in lung developmental or regenerative pathways may also contribute to impaired tissue repair in emphysema (15). We hypothesized that in emphysema, LMSCs are impaired in their function due to intrinsic defects and/or as a consequence of their interaction with the damaged microenvironment, thereby contributing to disturbed tissue repair in emphysema. To test this, we investigated potential differences between control- and COPD-derived LMSCs in differentiation, proliferation, the expression profile of growth factors that support tissue regeneration, for example, fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), WNT ligands, and ECM components, such as collagen I, fibronectin, and elastin. We also examined whether growth within an emphysematous lung tissue scaffold hampers their function, by seeding control- and COPD-derived LMSCs on control and emphysema decellularized lung tissue scaffolds.

MATERIALS AND METHODS

Subjects

Lung tissue was derived from a total of 18 patients with emphysema with GOLD stage III–IV COPD undergoing lung transplantation or lung volume reduction surgery and from leftover lung material from 27 non-COPD controls undergoing tumor resection surgery. See Table 1 for patient characteristics. Tissue was collected distant from the tumor. Tissue was checked for abnormalities by an experienced pathologist, and, if indicated, excluded from our study. The study protocol was consistent with the Research Code of the University Medical Center Groningen (https://www.umcg.nl/EN/Research/Researchers/General/ResearchCode/Paginas/default.aspx#:~:text=The%20UMCG%20Research%20Code%20provides%20researchers%20with%20guidelines,act%20in%20accordance%20with%20its%20rules%20of%20conduct) and the National Ethical and Professional Guidelines ("Code of Conduct; Dutch Federation of Biomedical Scientific Societies," http://www.federa.org).

Table 1. *Characteristics of subjects included in the study*

	Control, $n = 18$	COPD GOLD III–IV, $n = 27$
Sex	13 F/5 M	16 F/11 M
Smoking habit		
Current	0	0
Former	8	27
Never	10	0
Age, yr	68 (41–82)	59 (45–74)
FEV1%pred	106 (80–129)	24 (12–87)
FEV1/FVC	77 (69–90)	29 (22–98)

For age, FEV1%pred and FEV1/FVS, group medians with ranges are shown. Exclusion criteria for subject inclusion in the study were the diagnosis of asthma, indications of lung infection, COPD GOLD stage classification of I or II, or abnormalities in tissue structure. COPD, chronic obstructive pulmonary disease; FEV1%pred, predicted value for forced expiratory volume in 1 s; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

LMSC Isolation and Culture

LMSCs were acquired from peripheral parenchymal lung tissue by culture from tissue explants in DMEM/F-12 medium (Gibco, Waltham, MA) on fibronectin-coated plates as described in the Supplemental Data (all Supplemental Material is available at https://doi.org/10.6084/m9.figshare.13860659).

In *passage 2*, cells were seeded in six-well plates for characterization, RNA isolation, and supernatant collection. Cells were grown for 2–3 days to ~90% confluence, serum-deprived overnight, and placed into fresh serum-free medium for 24 h. Supernatants were stored for later analysis and the cells were lysed in TRI reagent (MRC, Cincinnati, OH) for RNA isolation, cDNA synthesis, and qPCR. RNA was isolated using the chloroform extraction method (16). cDNA was synthesized with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) in accordance to the manufacturer's instructions. qPCR was performed as described in the Supplemental Data (see Supplemental Tables S2 and S3 for the used primers).

Characterization of LMSCs

Expression of surface markers was measured by flow cytometry analysis using the BD FACSCalibur (BD Bioscience, Franklin Lakes, NJ) (see Supplemental Table S1 for antibodies and Supplemental Fig. S1 for gating strategy). Colony-forming-unit (CFU) potential and differentiation into adipocytes and osteoblasts were performed as described in the Supplemental Data. Metabolic activity was assessed by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay.

ELISA

FGF10, HGF, and decorin proteins were measured in cell-free supernatants using FGF10 ELISA kit (Aviva System Biology, San Diego, CA), HGF Duoset ELISA system (DY294, R&D Systems, Abingdon, UK), and Decorin Duoset ELISA system (DY143, R&D Systems, Abingdon, UK), respectively, in accordance with their respective manufacturer's instru-ctions.

Decellularization of Human Lung Tissue

Decellularized lung tissue scaffolds were generated from three patients with GOLD stage IV COPD with emphysema and three nonemphysema controls. Lung tissue blocks (~3 cm^3) were decellularized using a multistep, multiliquid protocol (17), adapted from previous studies (18) as described in the Supplemental Data, and subsequently 1 cm^3 pieces were prepared. Successful decellularization of lung tissue was assessed via hematoxylin-eosin (H&E) and DAPI staining, and DNA gels, showing that no residual cells were present

(Supplemental Fig. S2 and Supplemental Table S5). Major ECM components such as elastin, collagens, and fibronectin were conserved, whereas several glycosaminoglycans were partly removed (Supplemental Fig. S3).

Reseeding of Decellularized Lung Scaffolds with LMSCs

Decellularized scaffolds (~1 cm^3 pieces) were reseeded with/without 250^10^3 COPD- or control-derived LMSCs in DMEM/F-12 + 10% FCS +1% P/S for 24 h with rotation at 37°C. Reseeded scaffolds were subsequently placed into 24-well plates in DMEM/F-12 + 10% FCS + 1% P/S + 1% amphotericin B and cultured for 1–2 wk, fixed in 4% formalin, embedded in paraffin, and sliced for histological assessment.

Histological and Immunohistochemical Assessment of Reseeded Decellularized Lung Scaffolds

Paraffin sections were processed as described in the Supplemental Data (see Supplemental Table S4 for details on the staining). In short, sections were stained with hematoxylin-eosin (H&E) and by immunohistochemistry for Ki-67, type I collagen, type III collagen, elastin, fibronectin, FGF10, and HGF. Thickening of alveolar septa was scored blinded in a semiquantitative manner, using H&E sections and a visual analog score (VAS) of the complete section. Quantification was performed according to reference images (score 1–4, see Supplemental Fig. S4) by two independent observers. Quantification of Ki-67, FGF10, and HGF positivity was performed using Fiji Color Deconvolution plugin in Image-J as described in detail in the Supplemental Data.

Statistics

The Mann–Whitney *U* test was used when testing for differences between two groups. The Kruskal–Wallis test with correction for multiple testing was used when multiple groups and conditions were compared. $P < 0.05$ was considered statistically significant.

RESULTS

Differences in MSC Characteristics between Control- and COPD-Derived LMSCs

First, we assessed the expression of MSC surface markers in accordance with the criteria of the International Society for Cellular Therapy (7). All tested LMSCs were positive for mesenchymal markers CD105, CD29, CD90, CD73, and CD44 and negative for hematopoietic lineage markers CD34, CD31, and CD45 (Supplemental Fig. S5). In addition, LMSCs expressed CD146 (Supplemental Fig. S5), which has been suggested to be specific for MSCs (19). When comparing three COPD-derived to three control-derived LMSC cultures, no differences were observed between their surface marker profiles (Supplemental Fig. S5).

Assessment of the ability of LMSCs to differentiate to demonstrate multipotency showed that 80% of all tested LMSC cultures were able to differentiate into either adipocytes, osteoblasts, or both (see Supplemental Fig. S6, *A* and *B*, for representative staining), without differences between control- and COPD-derived LMSCs. CFU assays demonstrated that 70% of all LMSCs cultures were able to form colonies (at least >5 colonies, which were defined as >50 cells expanded from a single cell). Again, no differences were observed between control- and COPD-derived LMSC cultures (ranging from 1 to 30 colonies, Supplemental Fig. S6, *C* and *D*). No positive or inverse correlations were observed between CFU and differentiation potential or differentiation toward either adipocytes or osteoblasts (data not shown).

Control- and COPD-derived LMSCs showed no significant difference in metabolic activity between groups (Supplemental Fig. S7), confirming similar proliferative potential.

Lower FGF10 and HGF mRNA Expression and Decorin Secretion in COPD-Derived LMSCs

When assessing messenger RNA (mRNA) expression profiles, we observed that the expression of the growth factors FGF10 and HGF was lower in COPD-derived compared with control-derived LMSCs (Fig. 1*A*). Although FGF10 protein levels were below the detection limit (data not shown), we confirmed lower HGF in COPD-derived LMSCs at the protein level (Fig. 1*B*). Expression of the other growth factors, that is, FGF2, FGF7, WNT5A (Fig. 1*A*), WNT3A, NOTCH1, and PDGF (Supplemental Fig. S8) as well as ECM molecules COL1A1, FN1, ELN, POSTN, and DCN (Fig. 1*C*) were not significantly different between COPD- and control-derived LMSCs. Of the ECM genes, DCN mRNA was the highest expressed. Decorin protein was also highly secreted, and, of interest, we observed lower levels in COPD-derived LMSCs (Fig. 1*D*). PDGFRB and the genes encoding cytoskeletal protein α-smooth muscle actin (ACTA2) and bone morphogenetic receptor type IA and II (BMPR1A and BMPR2) were not differentially expressed (Supplemental Fig. S8). No mRNA expression of the anti-inflammatory cytokine IL10 and growth factor FGF19 was detected.

COPD- and Control-Derived LMSCs Show No Impairments in Engraftment, Proliferation, and/or Survival and Functionality on Decellularized Control Lung Tissue Scaffolds

To assess if COPD-derived LMSCs are impaired in their interactions with the ECM microenvironment, LMSCs were seeded on decellularized scaffolds of control lung tissue and cultured for 1–2 wk. No differences were observed between the groups when visually assessed by H&E staining (Fig. 2*A*). Ki-67 staining was analyzed to compare the ability of LMSCs to survive and proliferate on the lung tissue scaffolds. Two weeks after reseeding, Ki-67 positivity was observed for both control- and COPD-derived LMSCs, which was even higher for COPD-derived LMSCs (Fig. 2, *B* and *C*). LMSCs were functional as observed by the ability to increase density of the matrix. We did not observe major macroscopic shape changes in the scaffolds, suggesting that the increase in alveolar septa thickness was not the result of ECM fiber contraction, but rather the deposition of new ECM. When quantifying changes in thickness of the alveolar septal regions, we observed that both control- and COPD-derived LMSCs significantly increased the density of the matrix by ~twofold, indicative of new ECM deposition, on reseeded scaffolds compared with the scaffolds without cells after 1 wk. We did not observe significant differences between the groups (Fig. 3*A*), in line with the lack of difference in ECM gene expression. After 2 wk, control- and COPD-derived LMSCs further increased the thickness of alveolar septa to a similar extent (Fig. 3*B*).

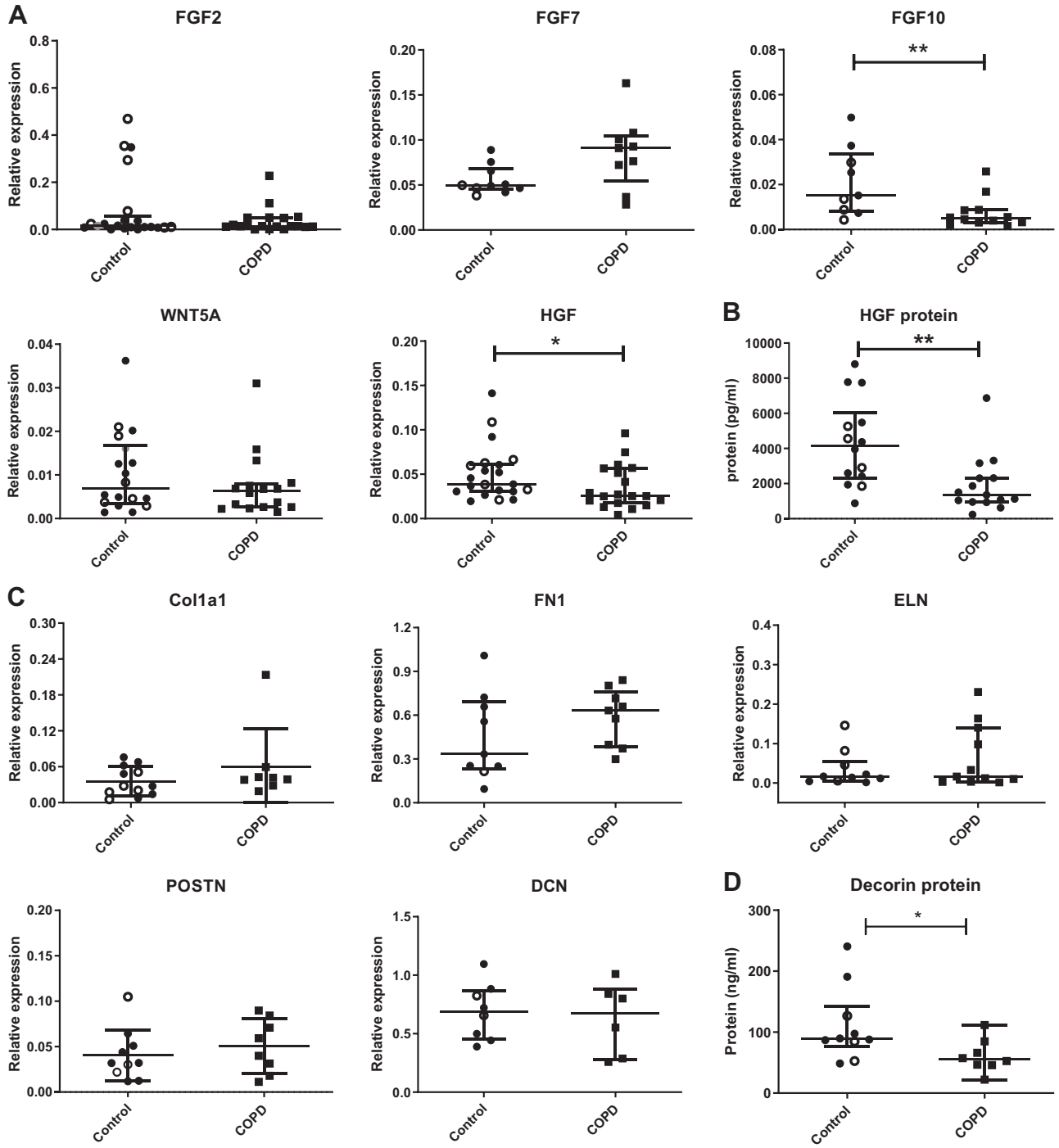

Figure 1. Lung-derived mesenchymal cells (LMSCs) from patients with COPD display lower levels of FGF10 mRNA, HGF mRNA and protein, and decorin protein than LMSCs from healthy controls. LMSCs were seeded in duplicates, grown to confluence, and serum deprived overnight. *A*: cells were harvested after 24 h and RNA was isolated, mRNA expression of growth factors *FGF2*, *FGF7*, *FGF10*, *WNT5A*, and *HGF* was assessed by qPCR and normalized for housekeeping gene *B2M* and expressed as $2^{-\Delta Ct}$ (Rel. Expression). *B*: HGF protein levels as determined by ELISA using the kit's standard curve in technical duplicates and averaged. *C*: mRNA expression of ECM components *COL1A1*, *FN1*, *ELN*, *POSTN*, and *DCN* was normalized for housekeeping gene *B2M*, represented as $2^{-\Delta Ct}$. *D*: DCN protein levels as determined by ELISA using the kit's standard curve. Medians ± interquartile range (IQR) are shown. Statistical significance was determined using the Mann–Whitney *U* test. $^{*}P < 0.05$; $^{**}P < 0.01$ between the indicated values. Open symbols indicate never smokers; closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; DCN, decorin; ECM, extracellular matrix; HGF, hepatocyte growth factor; mRNA, messenger RNA; Rel. Expression, relative expression.

To assess the composition of the newly formed matrix, the scaffolds were stained for collagens, fibronectin, and elastin. We observed that the thickened matrix of lung tissue scaffolds reseeded with LMSCs from both controls and patients with COPD contained collagen I and III, elastin, and fibronectin (Fig. 4). To assess whether the reseeded LMSCs were able to produce growth factors, we investigated the presence of FGF10 and HGF. The positivity for both growth factors was

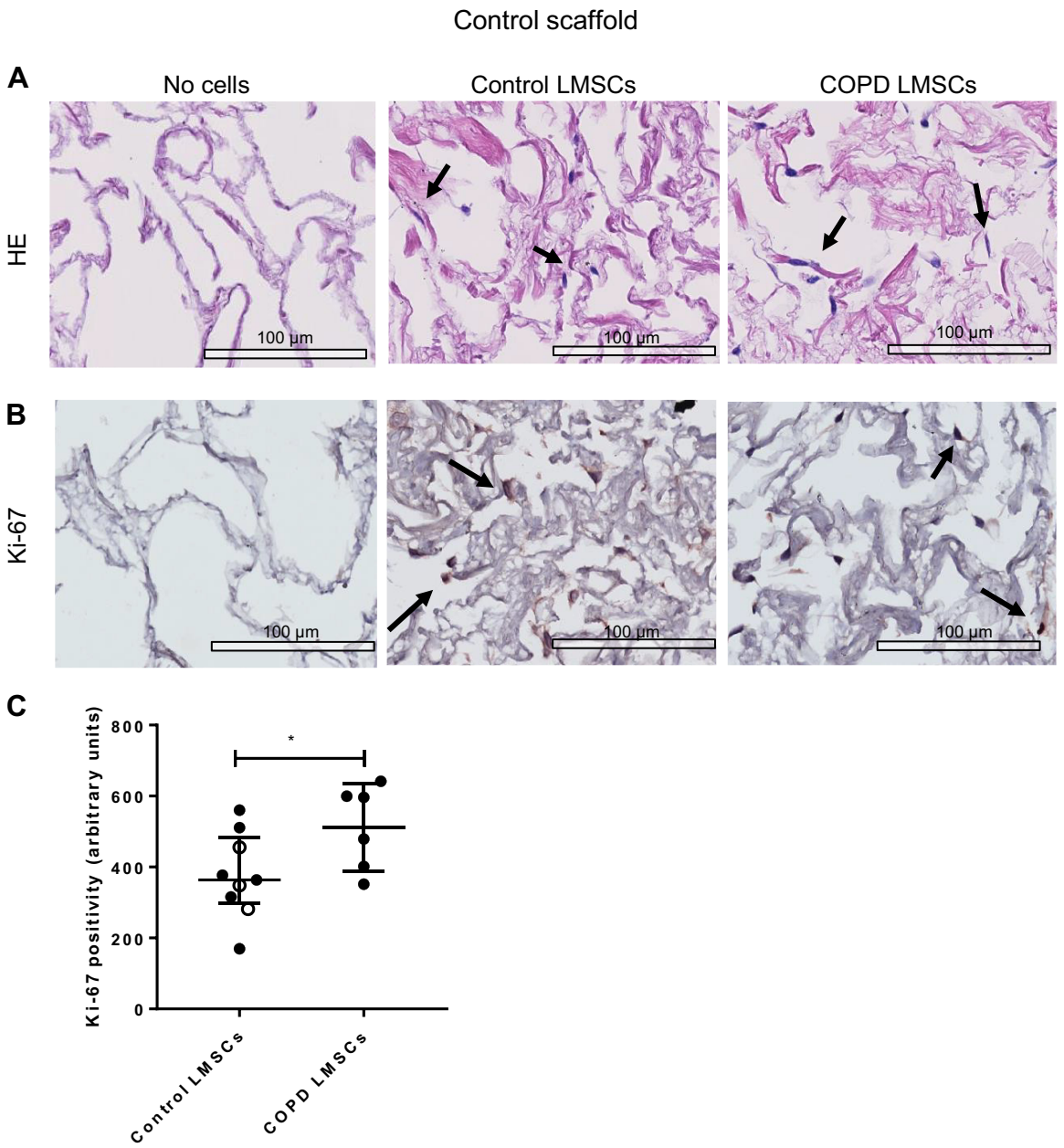

Figure 2. COPD-derived LMSCs do not show abnormalities in engraftment or proliferation compared with control-derived LMSCs when seeded on control decellularized lung tissue scaffolds. Decellularized lung scaffolds from controls were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. *A*: control-derived LMSCs and COPD-derived LMSCs stained with hematoxylin and eosin (HE), where pink color represents extracellular matrix and blue color represents cells as indicated by arrows. *B*: control-derived LMSCs and COPD-derived LMSCs stained for Ki-67 (as indicated by arrows) with hematoxylin counterstaining. All images are representative for their respective groups. The white bar represents a scale of 100 μm. *C*: quantification of number of Ki-67 positive pixels (red) per total number of nuclear pixels (blue) in images processed using Fiji color deconvolution. Medians ± IQR are shown. Statistical significance was determined using the Mann–Whitney *U* test. $*P < 0.05$. Open symbols indicate never smokers, and closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; IQR, interquartile range; LMSCs, lung-derived mesenchymal cells.

significantly higher in the reseeded than in the unseeded scaffolds after 2 wk, but was not different between scaffolds reseeded with either control- or COPD-derived LMSCs (Fig. 5).

No Impairments in Cell Numbers and Function of LMSCs Seeded on Emphysematous Lung Tissue Scaffolds

To assess whether an emphysematous microenvironment reduces cell numbers or impairs the function of COPD- and/or control-derived LMSCs, control- or COPD-derived LMSCs were cultured on scaffolds from emphysematous decellularized lung tissue. No differences were observed in cell numbers (control- or COPD-derived) at 1 wk (data not shown) or 2 wk of reseeding (Fig. 6*A*) on control and emphysematous lung tissue scaffolds. Control- and COPD-derived LMSCs (Fig. 6, *B* and *C*) showed comparable K_i-67 positivity when seeded on normal and emphysematous scaffolds (Fig. 6*C*).

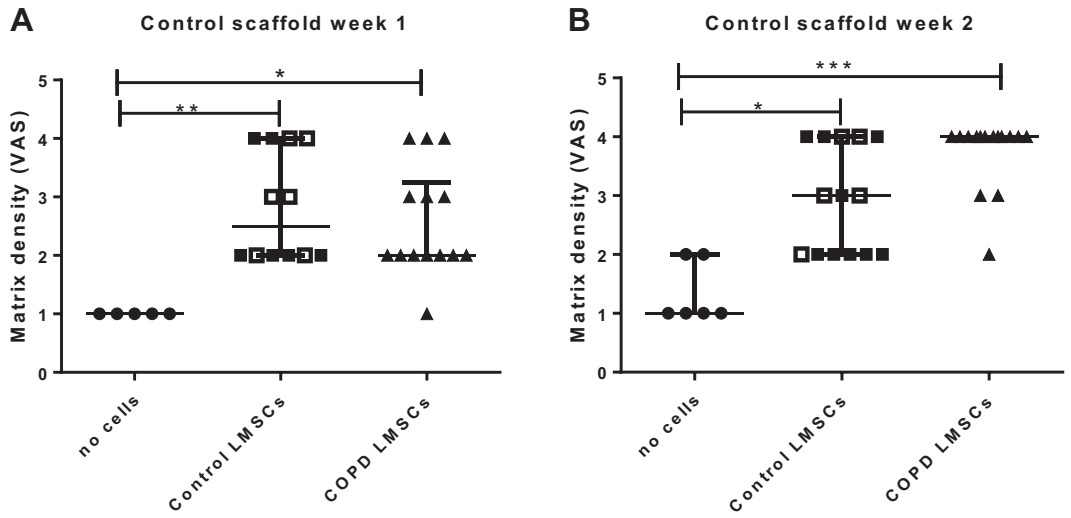

Figure 3. COPD- and control-derived LMSCs do not differ in their ability to increase the density of the matrix when seeded onto control decellularized lung tissue scaffolds. Decellularized control lung tissue scaffolds were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Paraffin sections were prepared, stained with H&E, and parenchymal thickening was assessed by visual analog score (VAS). *A*: VAS scores of decellularized scaffolds after 1 wk of culture without cells, with control-derived LMSCs or with COPD-derived LMSCs. *B*: VAS scores of decellularized scaffolds after 2 wk of culture without cells, with control-derived LMSCs or with COPD-derived LMSCs. Medians ± interquartile range (IQR) are shown. Statistical significance was determined using the Mann–Whitney *U* test. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ between the indicated values. Open symbols indicate never smokers, and closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; H&E, hematoxylin-eosin; LMSCs, lung-derived mesenchymal cells.

On the emphysematous scaffolds, LMSCs (both control- and COPD-derived) induced thickening of the alveolar septa to a similar extent as observed on the control tissue scaffolds, although the increase in parenchymal thickening was not significant with either control- or COPD-derived LMSCs at *week 1* (Fig. 7, *A* and *B*). In addition, the composition of the newly formed ECM (type I and III collagen, elastin, and fibronectin) was similar for control and emphysematous scaffolds (Supplemental Fig. S9). No significant differences were observed in FGF10 and HGF positivity between the control- and COPD-derived LMSCs at 2 wk after reseeding onto the emphysematous scaffolds (Fig. 8). Furthermore, no significant differences were observed in positivity for both FGF10 and HGF between reseeded normal and emphysematous scaff-olds.

Together, these results indicate that culture of LMSCs on emphysematous scaffolds does not impair their ability to engraft, proliferate, and/or survive, nor does the emphysematous environment significantly reduce the deposition of matrix and the proportion of growth factor-producing cells compared with growth on normal tissue scaffolds.

DISCUSSION

We hypothesized that LMSCs from patients with COPD are impaired in their ability to support tissue repair and show, for the first time, that LMSCs from patients with COPD express lower mRNA levels of HGF and FGF10 and secreted lower decorin levels than control-derived LMSCs. No differences were found in the expression of other growth factors, ECM genes, or other characteristics. Furthermore, control- and COPD-derived LMSCs had similar capacities to engraft and proliferate on control lung tissue. In line with the lack of difference between control- and COPD-derived mRNA expression of ECM genes, no differences were observed in their ability to deposit new matrix on decellularized normal lung tissue scaffolds. When seeded on emphysematous scaffolds, control- and COPD-derived LMSCs were equally able to engraft, proliferate, and/or survive as well as deposit ECM as on control tissue scaffolds. This indicates that the interaction with the emphysematous ECM-environment does not hamper the potential of LMSCs, at least not with respect to the engraftment, survival/proliferation, and deposition of matrix.

The lower production of growth factors in COPD-derived LMSCs, HGF in particular, may contribute to the impairment of alveolar epithelial repair in COPD. In line with our data, Kanazawa et al. (20) found reduced protein levels of HGF in the peripheral epithelial lining fluid of patients with COPD when compared with non-COPD controls. Importantly, HGF expression was shown to positively correlate to physical lung parameters such as alveolar diffusion capacity in the patients with COPD. We now show that LMSCs are a potential source of HGF. HGF has been shown to play an important supportive role in the repair of alveolar epithelial cells, increasing alveolar epithelial DNA synthesis in a mouse model of acute lung injury (21). Similar findings were observed by others, including by Wang et al. (22), showing that HGF increases alveolar epithelial organoid formation in vitro. Another study demonstrated protective effects of HGF on cigarette smoke extract-induced apoptosis of human bronchial epithelial cells in vitro (23). In addition, Kennelly et al. (24) demonstrated that the cytoprotective effects of BM-MSCs on alveolar epithelial cells in a murine model of COPD are at least, in part, mediated by HGF production. Similarly, Nita et al. (25) found that BM-MSC-conditioned medium increases alveolar epithelial repair in vitro at least partially through HGF. A mouse model showed improved

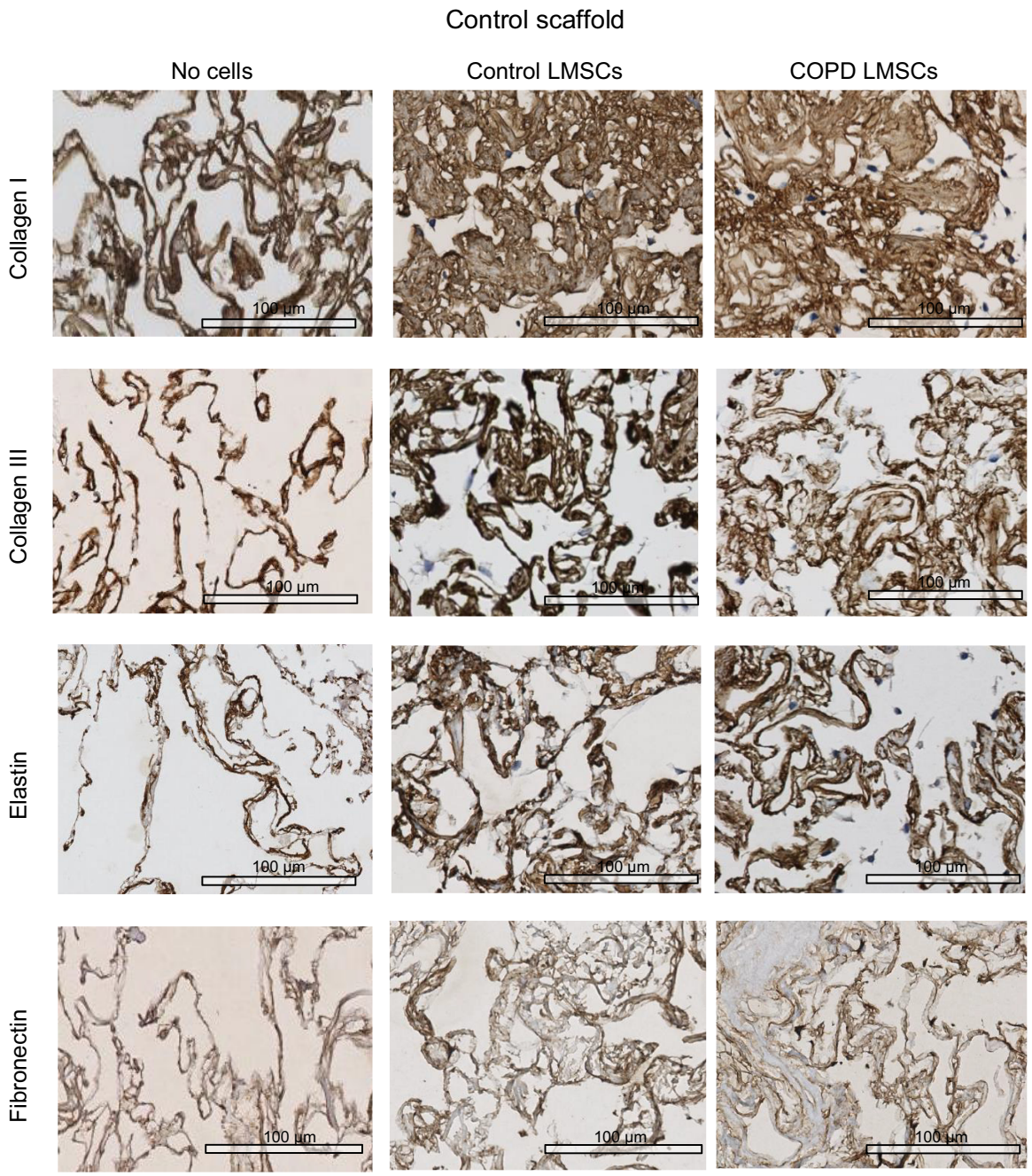

Figure 4. Composition of extracellular matrix in decellularized lung tissue scaffolds reseeded with COPD and control-derived LMSCs. Decellularized control tissue lung scaffolds were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Paraffin sections were prepared and stained for type I collagen, type III collagen, elastin, and fibronectin. All images are representative for their respective groups ($n = 6$ for unseeded scaffolds, $n = 15$ for scaffolds seeded with control LSMCs, $n = 15$ for scaffolds seeded with COPD LMSCs). The white bar represents a scale of 100 µm. COPD, chronic obstructive pulmonary disease; LMSCs, lung-derived mesenchymal cells.

protection from ischemic/reperfusion lung damage when HGF was overexpressed in BM-MSCs compared with the administration of wild-type BM-MSCs (26). Thus, the impaired HGF production in COPD-derived LMSCs may be important for the dysregulated repair in emphysematous lung tissue.

FGF10 was also lower expressed in COPD-derived LMSCs and is also thought to be a critical growth factor in the lungs. Many studies have shown a crucial role for FGF10 in lung developmental processes, which are often reactivated in the adult lung during lung tissue repair (27, 28). Min et al. (29) showed that *Fgf10*-knockout mice did not develop lungs, which was accompanied by lethality after birth. Ramasamy et al. (27) expanded on this *Fgf10*-knockout model by generating partial knockout mice, showing that reduced levels of *Fgf10* lead to severe lung hypoplasia, enlargement of the distal airways, and a specific reduction in epithelial cells expressing type II marker surfactant B. Besides LMSCs being

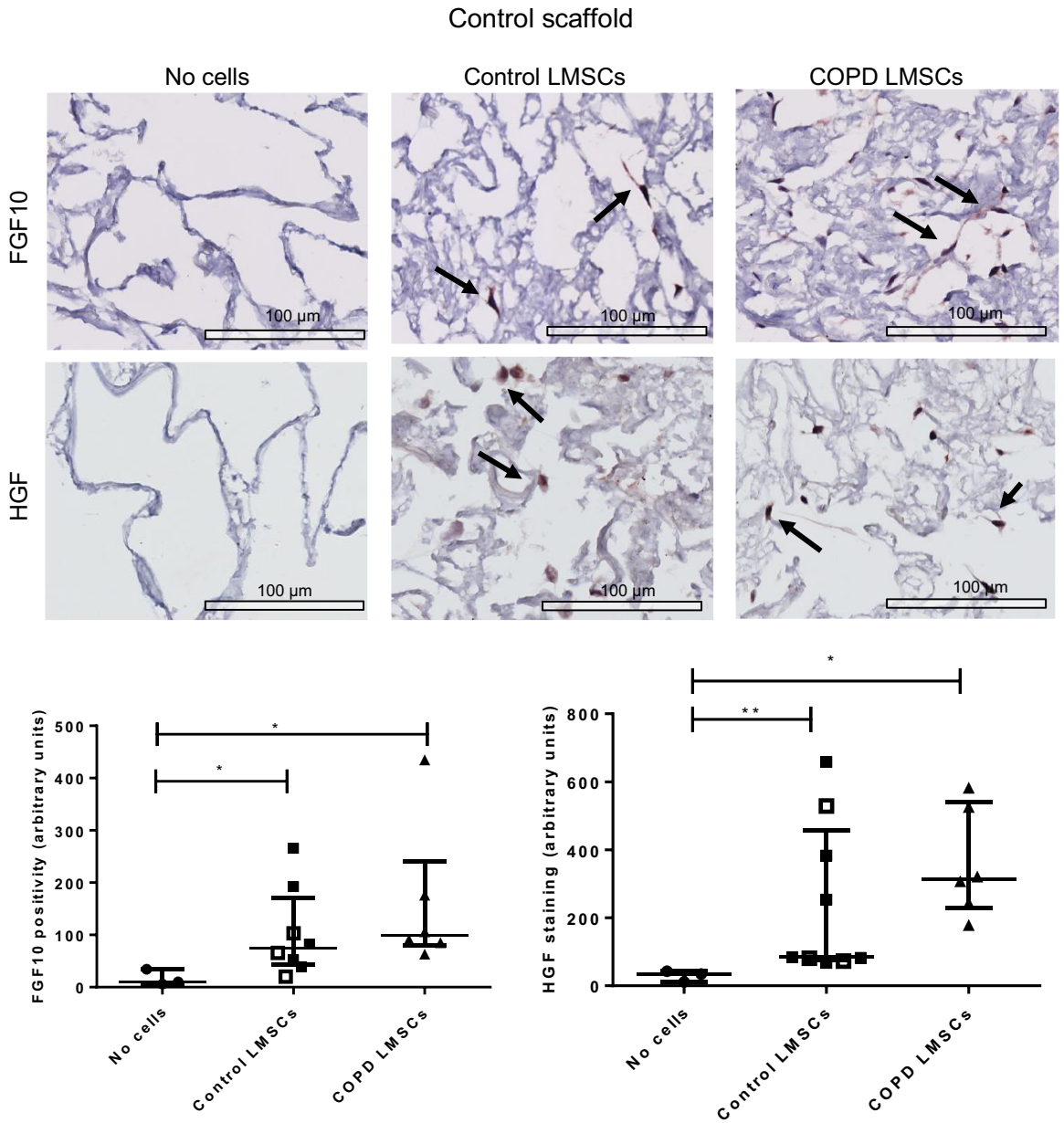

Figure 5. Similar expression of growth factors FGF10 and HGF in decellularized control lung tissue scaffolds reseeded with COPD and control-derived LMSCs. Decellularized control tissue lung scaffolds were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Paraffin sections were prepared and stained for FGF10 and HGF (NovaRED, as indicated by arrows) and counterstained with hematoxylin. All images are representative for their respective groups. White bar represents scale of 100 µm. Medians ± IQR are shown of the quantification of number of FGF10 and HGF positive pixels (red) per total number of nuclear pixels (blue) in images processed using Fiji color deconvolution. Statistical significance was determined using the Kruskal–Wallis test with correction for multiple testing. *$P < 0.05$; ** $P < 0.01$ between the indicated values. Open symbols indicate never smokers, closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; HGF, hepatocyte growth factor; IQR, interquartile range; LMSCs, lung-derived mesenchymal cells.

a source of FGF10, FGF10 has also been shown to function as an LMSC mobilizer and recruiter (30). Thus, reduced expression of this factor in the stromal cell population of patients with COPD could act in a negative feedback loop, influencing not only the cell's ability to orchestrate local repair responses at the site of injury but also leading to reduced migration of supportive cells to the damaged site.

In addition to HGF and FGF10, other MSC-derived growth factors have been implicated in lung development and alveolar epithelial repair, for example, FGF7, WNT ligands, and Notch ligands. Furthermore, the deposition of ECM components is crucial in tissue repair responses, for example, elastin has been recognized for its role in alveolar development (31). We did not find differences in these factors and elastin expression between COPD- and control-derived LMSCs. However, we did find lower secretion of ECM molecule decorin by COPD-derived MSCs. Its mRNA expression was not different between groups, indicating regulation at the posttranscriptional level. Decorin is a proteoglycan that binds to collagen fibrils, providing structural support. In

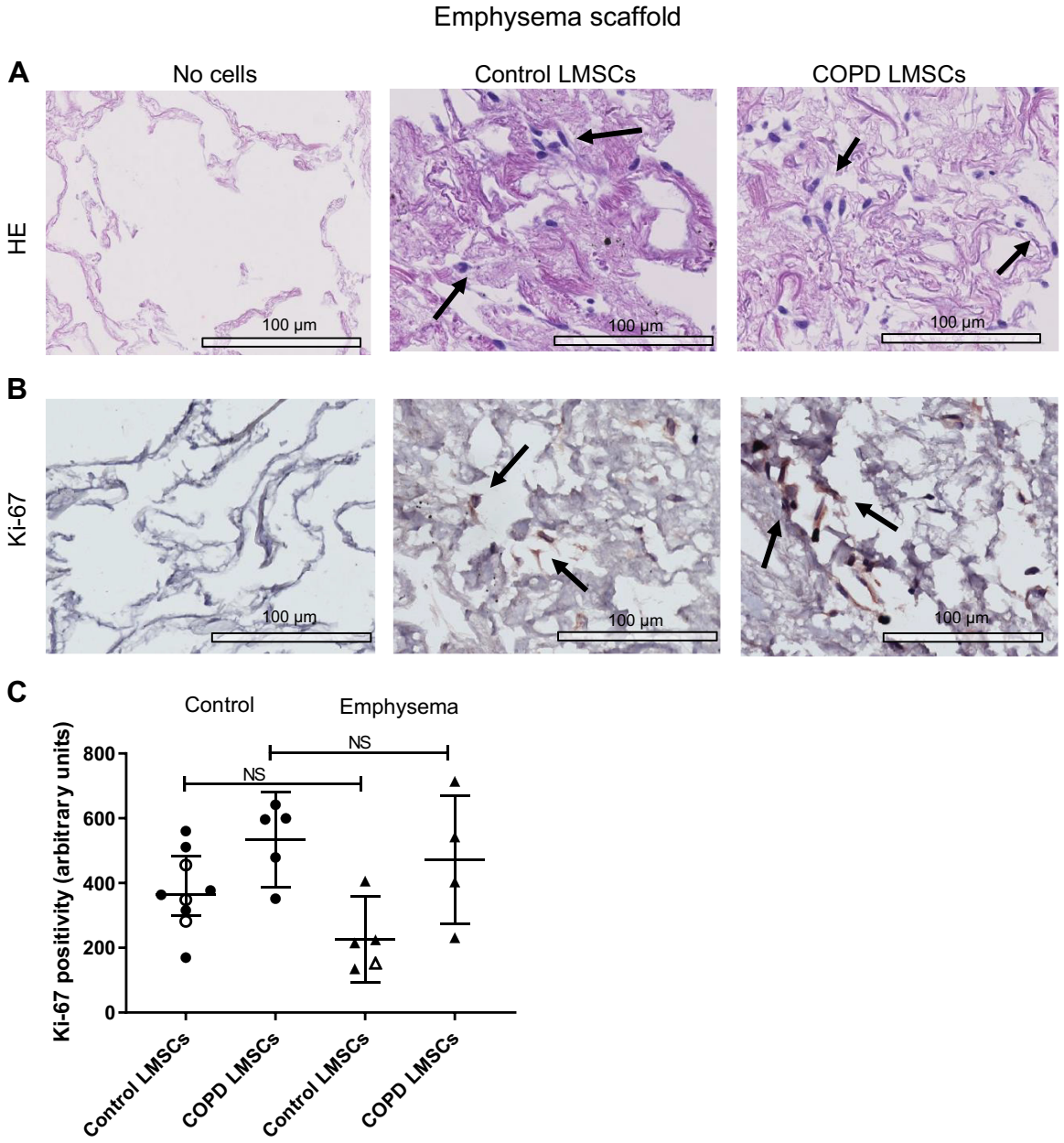

Figure 6. COPD-derived and control-derived LMSCs do not show abnormalities in engraftment or proliferation when seeded on emphysematous decellularized lung tissue scaffolds. Decellularized lung scaffolds derived from emphysematous lung tissues were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Paraffin sections were prepared. *A*: control-derived LMSCs and COPD-derived LMSCs stained with H&E, where pink color represents extracellular matrix and blue color represents cells as indicated by arrows. *B*: control-derived LMSCs and COPD-derived LMSCs stained for Ki-67 (as indicated by arrows) with hematoxylin counterstaining. All images are representative for their respective groups. The white bar represents a scale of 100 µm. *C*: quantification of number of Ki-67 positive pixels (red) per total number of nuclear pixels (blue) in images processed using Fiji color deconvolution. Medians ± IQR are shown. Statistical significance was determined using the Kruskal–Wallis test with correction for multiple testing. Open symbols indicate never smokers, and closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; H&E, hematoxylin-eosin; HGF, hepatocyte growth factor; IQR, interquartile range; LMSCs, lung-derived mesenchymal cells; NS, not significant.

addition, decorin can bind various growth factors and their receptors, regulating their activity. Our findings are in line with lower protein levels of proteoglycans, including decorin in emphysema (6), and it will be of interest to study the role of decorin in aberrant lung tissue repair response in the future.

We did not find an impairment in proliferative or clonogenic responses as a measure of LMSC stemness, nor in the ability to differentiate into multiple lineages. These results indicate that abnormalities in these aspects of reparative support by LMSCs may not contribute to the disrupted alveolar epithelial repair seen in emphysema. However, we cannot exclude that the factors measured at the mRNA level are not altered at protein level. Nevertheless, the lack of abnormalities in expression of various ECM genes was supported at the protein level when reseeding the LMSC on decellularized

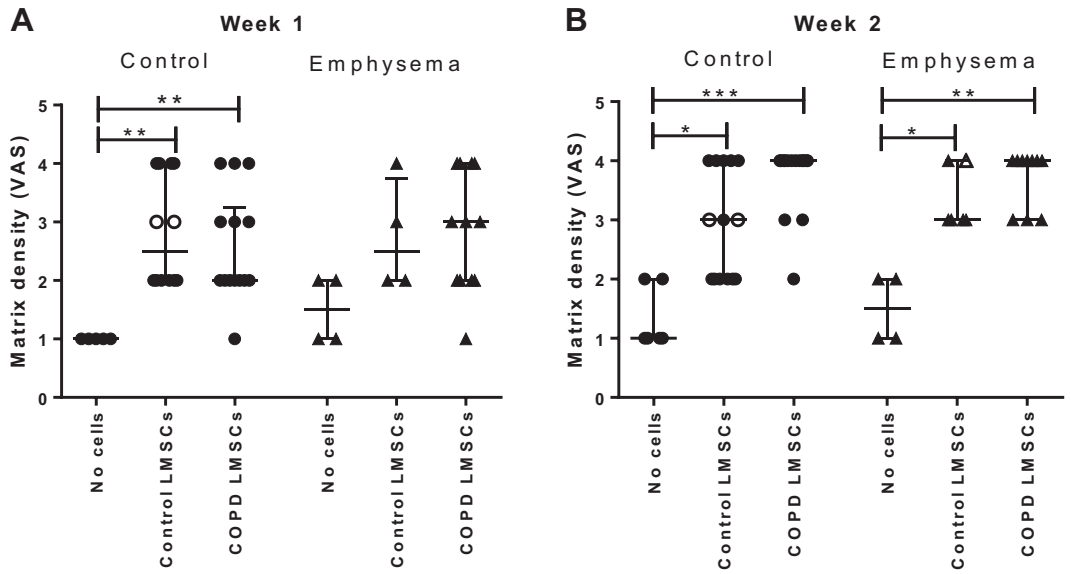

Figure 7. Emphysematous scaffolds do not change the ability of LMSCs to increase matrix density compared with scaffolds from normal lung tissue. Decellularized emphysematous tissue lung scaffolds were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Paraffin sections were prepared, stained with H&E, and parenchymal thickening was assessed by visual analog score (VAS). *A*: VAS scores of decellularized scaffolds after 1-wk culture without cells, with control-derived LMSCs or with COPD-derived LMSCs. *B*: VAS scores of decellularized scaffolds after 2 wk of culture without cells, with control-derived LMSCs or with COPD-derived LMSCs. Significance was determined using the Kruskal–Wallis test with correction for multiple testing. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ between the indicated values. Open symbols indicate never smokers, and closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; H&E, hematoxylin-eosin; HGF, hepatocyte growth factor; LMSCs, lung-derived mesenchymal cells.

lung tissue scaffolds, showing no impairments in COPD-derived LMSCs. Furthermore, being present in a destructed lung tissue environment did not impair LMSC's ECM production. However, other factors within the microenvironment of COPD lungs could impact on the ability to produce ECM, for example, soluble ECM components or ECM breakdown products washed out during the decellularization process, the presence of oxidative stress, a proinflammatory milieu, and/or cross talk with other cells (possibly senescent cells). Broekman et al. (32) showed that exposure of human BM-MSCs to the inflammatory cytokines TNF-α or IL-1β increased their capacity to support epithelial wound closure. For future studies, it would therefore be of interest to investigate the effects of inflammatory mediators and/or cigarette smoke components on LMSC repair responses, specifically in the context of our reseeded decellularized scaffold models, representing the native ECM environment.

Because the ECM environment instructs cellular function and is disrupted in emphysematous lung parenchyma, we hypothesized that the interaction of LMSCs with an emphysematous microenvironment may impair their function (33). We did not observe impairments in the ability to engraft, grow, or function on emphysematous scaffolds compared with normal lung tissue. In contrast to our findings, Wagner et al. (34) have previously shown that bone marrow-derived MSCs are less able to survive on emphysematous lung tissue scaffolds compared with normal scaffolds. Although the emphysematous scaffolds in their study supported initial engraftment and cell growth, cells did not survive beyond a week on emphysematous scaffolds, whereas cells survived for up to 1 mo in normal scaffolds. This defect was attributed to disrupted three-dimensional (3-D) structure of the emphysematous lungs. One of the most obvious differences between Wagner's and our study is that we used lung transplantation or resection tissue, whereas Wagner's study made use of cadaveric material, with the cause of death being pneumonia, cardiac failure, neurological, or other. Emphysematous lung tissue could potentially be more fragile and vulnerable to disruption of architecture upon death, which could offer an explanation for the impaired cell survival on emphysematous scaffolds observed in Wagner's study.

In the emphysematous lungs of patients, particularly the expression of ECM components elastin and various glycosaminoglycans, including decorin and heparan sulfate are reduced (6, 35, 36). Because various glycosaminoglycans were partly lost during the decellularization process, we cannot exclude that differences in proteoglycans would contribute to abnormalities in LMSC responses in the emphysematous matrix, especially as various studies have shown that glycosaminoglycans retain and activate important growth factors such as HGF and FGF2 (37, 38). Although beyond the scope of the current manuscript, further studies will be performed to explore this. Another limitation of the current study is that we did not assess the functionality of cells at earlier time points after reseeding and may thus have missed potential differences. Moreover, we only used a single cell type in the recellularization studies, whereas the interaction of LMSCs with alveolar epithelial cells, endothelial cells, and/or immune cells will be of interest to further elucidate pathological features. We recommend this for future studies. Furthermore, the smoking status of the donors may have been a confounding factor in our studies. All patients with COPD were former smokers, whereas a

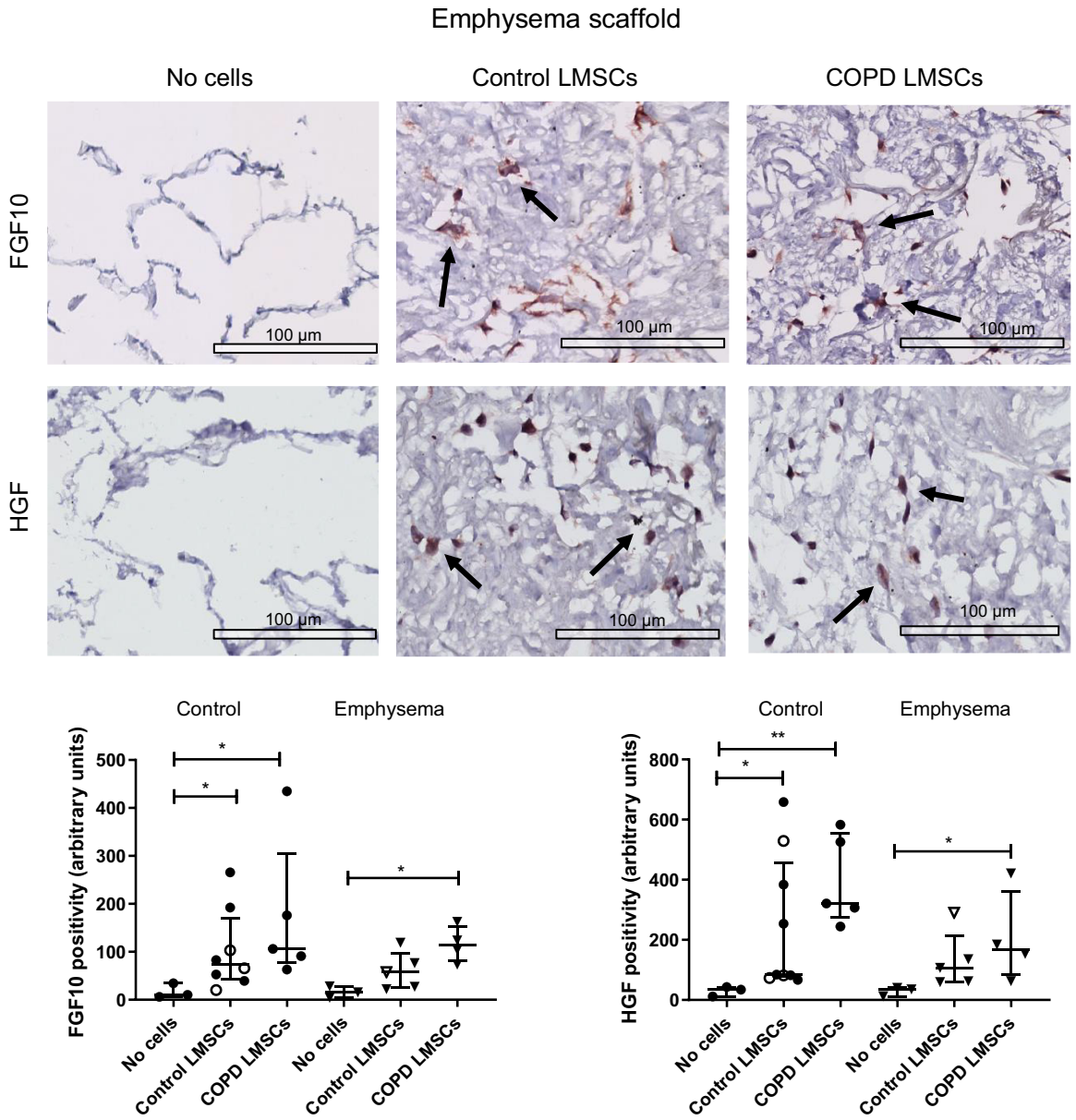

Figure 8. Similar expression of growth factors HGF and FGF10 in decellularized emphysematous lung tissue scaffolds reseeded with COPD and control-derived LMSCs. Decellularized emphysematous tissue lung scaffolds were reseeded with/without COPD or control-derived LMSCs and cultured for 2 wk. Scaffolds without cells were treated identically to the cell-seeded scaffolds. Paraffin sections were prepared and stained for HGF and FGF10 (NovaRED, indicated by arrows) and counterstained with hematoxylin. All images are representative for their respective groups. The white bar represents a scale of 100 µm. Medians ± IQR are shown of the quantification of number of FGF10 and HGF positive pixels (red) per total number of nuclear pixels (blue) in images processed using Fiji color deconvolution. Kruskal–Wallis test with correction for multiple testing. *$P < 0.05$; **$P < 0.01$ between the indicated values. Open symbols indicate never smokers, and closed symbols indicate former smokers. COPD, chronic obstructive pulmonary disease; IQR, interquartile range LMSCs, lung-derived mesenchymal cells.

number of the non-COPD controls were never smokers. However, as can be appreciated from our results, never smokers were not outliers in any of the read-outs and removal of the never smokers from these analyses did not affect any of the outcomes.

Upon the decellularization process, FGF10 and HGF were hardly detected on the lung tissue scaffolds. Both factors were expressed by LMSCs reseeded on the scaffolds. Positivity was not different between scaffolds reseeded with control- and COPD-derived LMSCs, reflective of an equal proportion of FGF10 and HGF-positive cells. Although immunohistochemistry does not allow for quantitative assessment of growth factor production per cell, this is not in line with the lower HGF protein levels in COPD compared with control-derived LMSCs at baseline. Therefore, it will be of interest in future studies to assess the ability of a healthy ECM environment to improve growth factor production by LMSCs and assess whether prolonged periods of culture in an emphysematous context (ECM and other factors) can lead to loss of HGF production.

In conclusion, our study indicates deficits in LMSCs from patients with COPD with respect to the expression of FGF10 and HGF, which may contribute to the impaired alveolar repair observed in patients with COPD. Future studies are warranted to assess the potential of therapeutic strategies in emphysema directed toward improvement of FGF10 and HGF production by LMSCs.

SUPPLEMENTAL DATA

Supplemental Tables S1–S5: https://doi.org/10.6084/m9.figshare.13860659.

Supplemental Figs. S1–S9: https://doi.org/10.6084/m9.figshare.13860659.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.M.L.W.K., W.F.D., T.H.v.K., N.H.T.t.H, and I.H.H. conceived and designed research; D.M.L.W.K., M.W., H.G.d.B, M.E.L., D.J.H., and T.B. performed experiments; D.M.L.W.K., M.W., H.G.d.B, M.E.L., D.J.H., T.B., and I.H.H. analyzed data; D.M.L.W.K., W.F.D., T.H.v.K., W.T., J.K.B., N.H.T.t.H, and I.H.H. interpreted results of experiments; D.M.L.W.K., M.W., H.G.d.B, M.E.L., D.J.H., and I.H.H. prepared figures; D.M.L.W.K. drafted manuscript; J.K.B., N.H.T.t.H, I.H.H., D.J.H., W.F.D., T.H.v.K., and W.T. edited and revised manuscript; D.M.L.W.K., M.W., H.G.d.B., M.E.L., D.J.H., T.B., W.F.D., T.H.v.K., W.T., J.K.B., N.H.T.t.H, and I.H.H. approved final version of manuscript.

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