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ORIGINAL ARTICLE

Perfluorocarbon solutions limit tubular epithelial cell injury and promote CD133$^+$ kidney progenitor differentiation: potential use in renal assist devices for sepsis-associated acute kidney injury and multiple organ failure

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ABSTRACT

Background. The renal assist device (RAD) is a blood purification system containing viable renal tubular epithelial cells (TECs) that has been proposed for the treatment of acute kidney injury (AKI) and multiple organ failure. Perfluorocarbons (PFCs) are oxygen carriers used for organ preservation in transplantation. The aim of this study was to investigate the effect of PFCs on hypoxia- and sepsis-induced TEC injury and on renal CD133$^+$ progenitor differentiation in a microenvironment similar to the RAD.

Methods. TECs were seeded in a polysulphone hollow fibre under hypoxia or cultured with plasma from 10 patients with sepsis-associated AKI in the presence or absence of PFCs and were tested for cytotoxicity (XTT assay), apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling assay, caspases, enzyme-linked immunosorbent assay, Fas/Fas Ligand pathway activation), mitochondrial activity, cell polarity [transepithelial electrical resistance (TEER)] and adenosine triphosphate production. The effect of PFCs on proliferation and differentiation of human CD133$^+$ progenitors was also studied.

Results. In the presence of PFCs, TECs seeded into the polysulphone hollow fibre showed increased viability and expression of insulin-like growth factor 1, hepatocyte growth factor and macrophage-stimulating protein. Plasma from septic patients induced TEC apoptosis, disruption of oxidative metabolism, alteration of cell polarity and albumin uptake, down-regulation of the tight junction protein ZO-1 and the endocytic receptor megalin on the TEC surface. These detrimental effects were significantly reduced by PFCs. Moreover, PFCs induced CD133$^+$ renal progenitor cell proliferation and differentiation towards an epithelial/tubular-like phenotype.

Conclusions. PFCs improved the viability and metabolic function of TECs seeded within a polysulphone hollow fibre and subjected to plasma from septic AKI patients. Additionally, PFCs promoted differentiation towards a tubular/epithelial phenotype of CD133$^+$ renal progenitor cells.

Keywords: acute kidney injury, oxygen carriers, renal assist devices, renal replacement therapy, sepsis

BACKGROUND

Acute kidney injury (AKI) affects ∼5% of long-term hospitalized patients [1]. AKI requiring renal replacement therapy (RRT) is associated with an increased risk of mortality, multiple organ failure and progression towards chronic kidney disease [1]. Sepsis, the systemic inflammatory response to infections, represents the leading cause of AKI in intensive care units (ICUs) [2–4]. The pathogenic mechanisms of sepsis-associated AKI are related to the ischaemic injury consequent to tissue hypoperfusion as well as to the direct detrimental activity of

circulating pro-apoptotic and inflammatory mediators on renal resident cells [3, 5]. We have previously demonstrated that plasma from septic patients induces apoptosis and functional alterations of glomerular and tubular epithelial cells (TECs) [6].

Several studies showed that extracorporeal blood purification techniques remove a broad range of harmful mediators mainly by convection or adsorption mechanisms [7]. Cell therapy represents a new approach for the treatment of sepsis-associated AKI [8, 9]. A bioartificial renal assist device (RAD) containing living renal tubular cells has been engineered demonstrating the adsorptive, metabolic and endocrine functions of the normal kidney [10]. The addition of RAD to conventional haemofiltration improved the outcomes of sepsis-associated AKI in preclinical models and clinical trials [11]. However, the clinical use of RAD is limited by the problems associated with large-scale cell culture, such as the preservation of long-term viability and the limited transport of nutrients and oxygen (O_2) [11, 12].

Perfluorocarbons (PFCs) are O_2 carriers used as organ preservation liquid in pancreatic islet transplantation with the aim of reducing hypoxia-induced apoptosis [12]. These molecules were initially developed to preserve highly corrosive uranium, as they are chemically and biologically inert: there is no evidence that any sort of molecular modification occurs under conditions of processing, storage and use [13]. Hence it is not surprising that PFC derivatives have been tested as haemoglobin substitutes and to improve the function of a hepatic bioreactor containing adult human liver cells [13, 14]. To date, one PFC molecule has been approved by international pharmaceutical agencies: Fluosol. Fluosol has been injected in >40 000 patients during cardiac angioplasty to increase myocardial oxygenation, prevent procedure induced ischemic episodes and preserve the ejection fraction [13, 15]. The use of PFCs has also been proposed to induce stem cell differentiation: high O_2 tension induced B-cell differentiation in rat embryonic pancreas [12, 16]. Moreover, other authors demonstrated that PFCs solubilize reactive oxygen species (ROS), thus acting as an unconventional scavenger [17]. Accordingly, we hypothesized that PFCs may improve RAD efficiency by two different mechanisms: (i) the induction and stabilization of the tubular cell phenotype within the hypoxic microenvironment of the bioreactor and (ii) the protection of RAD cells from ROS and inflammatory mediators involved in sepsis-associated cell injury. Thus the aim of this study was to evaluate *ex vivo* the protective effects of PFCs on TECs and on $CD133^+$ renal progenitors challenged by hypoxia and septic plasma.

MATERIALS AND METHODS

Patients

We enrolled 10 patients in the study from the Intensive Care Unit of the San Bortolo Hospital, Vicenza, Italy. Inclusion criteria were the presence of septic shock in accordance with the criteria defined by the International Consensus Definitions for Sepsis and Septic Shock [18] and the presence of AKI [inclusion at least in the injury group of risk, injury, failure, loss, end-stage renal disease (RIFLE) criteria according to serum creatinine and/or urine output] [19]. Exclusion criteria were age <18 years, solid organ or bone marrow transplantation, haemorrhagic dysfunction, thrombophilia, previous evidence of end-stage chronic kidney disease, glomerulonephritis or collagenopathies. Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores assessed the severity of illness at the moment of ICU admission and at the start of RRT. Outcome and renal function [estimated glomerular filtration rate (eGFR)] were evaluated 28 days after study admission or at ICU discharge. Plasma samples were drawn from all patients at the beginning of RRT. Informed consent was obtained according to the Declaration of Helsinki; the Internal Review Board of the San Bortolo Hospital authorized the study. Plasma from 10 age- and gender-matched healthy volunteers was used as a control for *in vitro* studies.

Cell isolation, characterization and culture

TECs and $CD133^+$ renal progenitors were isolated from adult human kidneys by magnetic cell sorting as previously described [20]. Briefly, primary cultures of human TECs were obtained from kidneys removed by surgical procedures from patients affected by renal carcinomas. $CD133^+$ renal progenitors were isolated from the tubular fraction by using the magnetic-activated cell sorting (MACS) system (Miltenyi Biotec, Auburn, CA, USA). Both cell types were seeded on collagen-coated polycarbonate transwell membranes (Corning, Cambridge, MA, USA) and cultured with Roswell Park Memorial Institute (RPMI) medium supplemented with 10% foetal calf serum and PFCs (FluoroMed, Round Rock, TX, USA) (dilution 50% RPMI, 50% PFCs) (Figure 1A). PFCs were pre-incubated with O_2 at 2 atm for 10 min [21]. For hypoxia experiments, TECs were cultured for 24 h into an airtight humidified chamber after flushing with a gas mixture containing 5% CO_2, 94% N_2 and 2% O_2 at 20 atm and 37°C for 5 min [22].

TEC culture within hollow fibres

TECs were seeded within the inner surface of a polysulphone hollow fibre (FX80 Fresenius Medical Care, Bad Homburg, Germany) in the presence or absence of PFCs (dilution 50% RPMI, 50% PFCs) in the inner part of the filter [23] (Figure 1B). After 48 h of culture, medium was removed for the determination of cell viability (XTT assay, Trevigen, Gaithersburg, MD, USA) and release of insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF) and macrophage-stimulating protein (MSP) (R&D Systems, Minneapolis, MN, USA) by enzyme-linked immunosorbent assay (ELISA). All experiments were performed in triplicate.

In vitro assays on TECs

TECs were incubated in the presence of septic plasma (10% dilution in RPMI), lipopolysaccharide (LPS; 30 ng/mL; Sigma Aldrich, St Louis, MO, USA) or inflammatory cytokines [20 ng/mL tumour necrosis factor (TNF)-α + 20 ng/mL (interferon (IFN)-γ; Sigma Aldrich]. Then 5×10^4 cells/well were incubated with appropriate agonists and 10 μM BrdU (Roche Diagnostics, Mannheim, Germany) or 250 μg/mL XTT in a medium lacking phenol red. The absorption values 405 nm (BrdU) or 450 nm (XTT) were measured in an automated spectrophotometer at different time points. The effect of PFCs and septic plasma on TECs

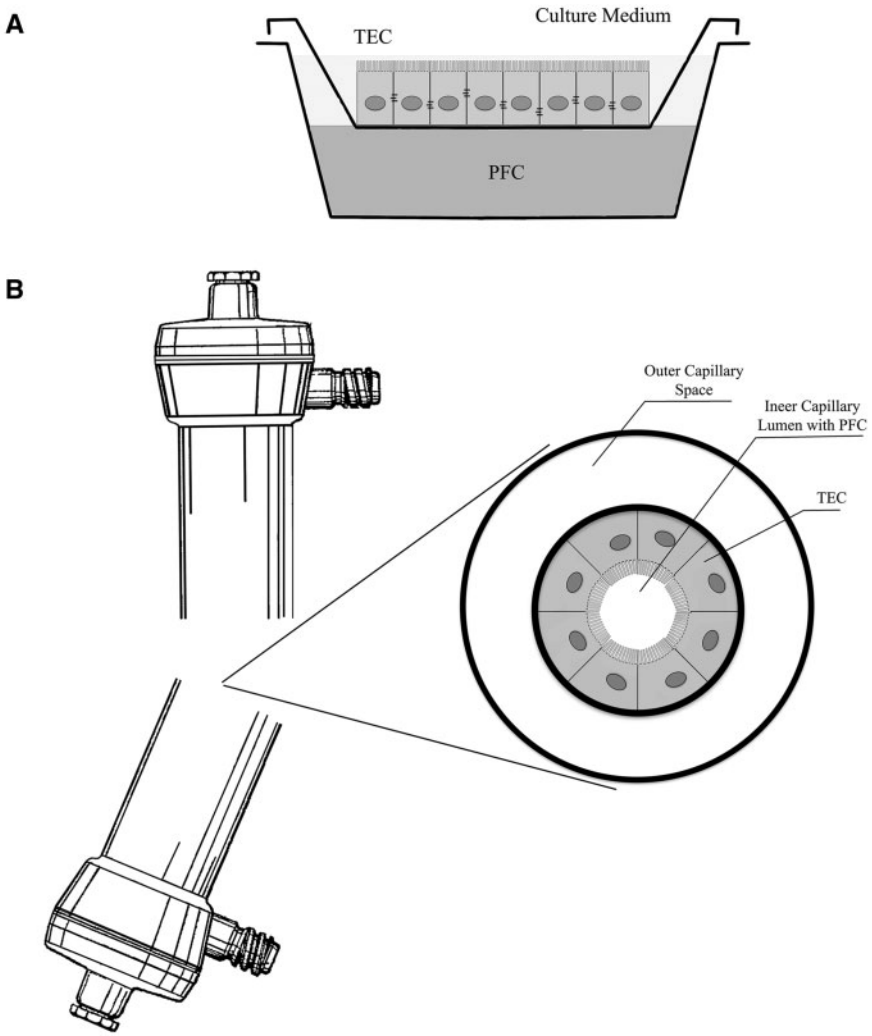

FIGURE 1: Schematic representation of the experimental models used in the study. (**A**) *In vitro* culture of TECs or CD133$^+$ renal progenitor cells seeded on collagen-coated polycarbonate membranes in the presence of PFCs. (**B**) *In vitro* culture of TECs injected within the inner part of a polysulphone hollow fibre in the presence of PFCs.

was investigated by cytotoxicity (XTT-based assay), apoptosis [terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay-ApopTag; Millipore, Temecula, CA, USA], ELISA for caspase-3, -8 and -9 activities (Millipore), cell polarity measured as transepithelial electrical resistance (TEER) and uptake of fluorescein-labelled albumin; all the protocols have been already described [22, 24]. Fluorescence-activated cell sorting (FACS) analysis for Fas and Fas ligand expression was performed after staining with phycoerythrin-conjugated specific primary antibodies (Biolegend, San Diego, CA, USA). Immunofluorescence studies for analysis of the mitochondrial protein PGC-1α, the tight junction protein ZO-1 and the endocytic receptor megalin were performed using specific primary antibodies (Santa Cruz Biotech, Santa Cruz, CA, USA) and Alexa Fluor 488–conjugated secondary anti-isotype antibodies (Life Technologies, Carlsbad, CA, USA) [6]. Nuclei were counterstained in blue with 2.5 μg/mL Hoechst. Mitochondrial membrane potential was assessed by the fluorescent dye MitoTracker Red 7513 (reduced chloromethyl-X-rosamine; Invitrogen) [25]. The ATP Assay Kit (Abcam, Cambridge, MA, USA) was used to quantify colorimetrically (optical density 570 nm) the total adenosine triphosphate (ATP) in TEC lysates. For all *in vitro* assays, cells were cultured for 24 h unless otherwise specified.

In vitro assay on CD133$^+$ human renal progenitor cells

The effect of PFCs on CD133$^+$ progenitor proliferation was tested by BrdU assay. Cell differentiation towards an epithelial/tubular phenotype was assessed by measuring TEER and by quantifying the number of tubular-like structures onto Matrigel-coated plates (Becton Dickinson, Franklin Lakes, NJ, USA) [26]. Immunofluorescence studies and FACS analysis for E-cadherin, megalin, alkaline phosphatase, aminopeptidase A, aquaporin-1 (AQP-1) and lipocalin-2 [neutrophil gelatinase-associated lipocalin (NGAL)] were performed with specific primary antibodies (all from Santa Cruz Biotech) and Alexa Fluor 488–conjugated secondary anti-isotype antibodies [6]. Nuclei were counterstained in blue with 2.5 μg/mL Hoechst.

Statistical analysis

All data are expressed as averages ± SD unless otherwise specified; experiments were performed in triplicate. Statistical analysis was performed by analysis of variance with the

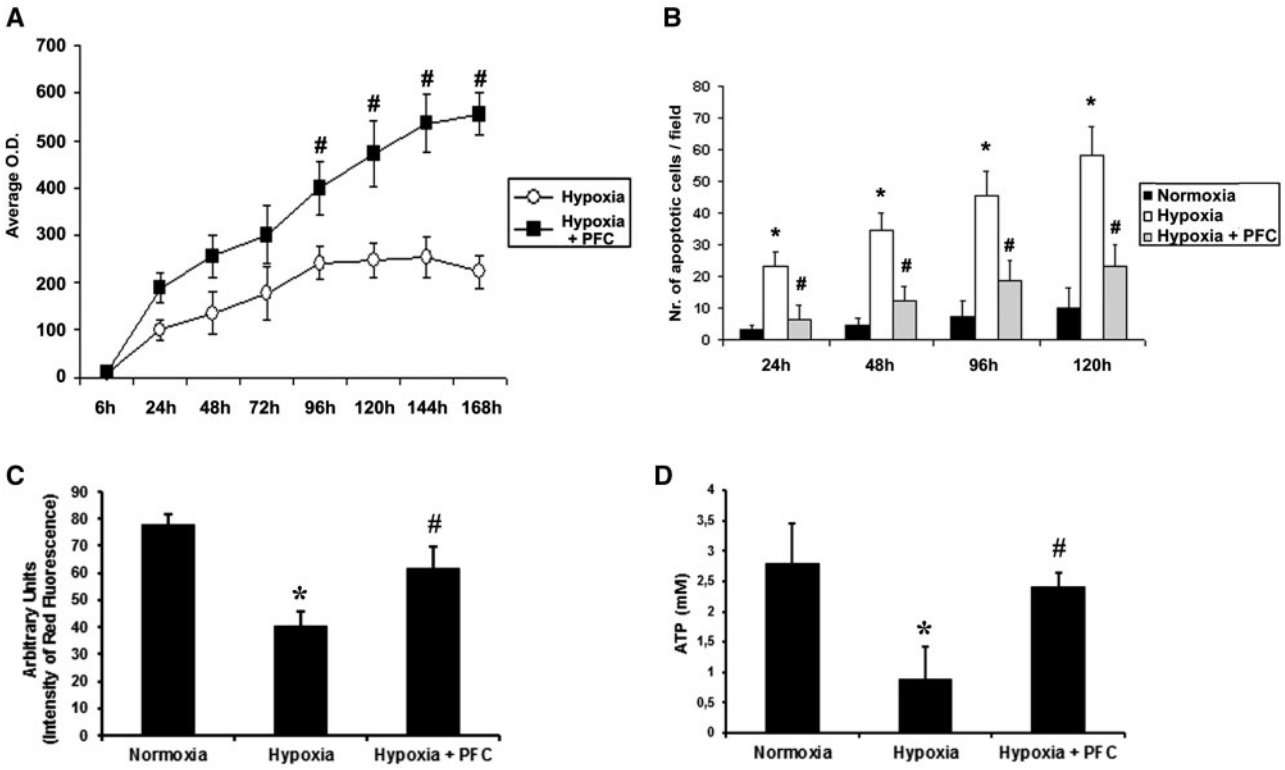

FIGURE 2: Effect of PFCs on viability, apoptosis and mitochondrial function of TECs seeded on collagen-coated polycarbonate membranes under hypoxic conditions. (**A**) Time-course analysis of viability (XTT-based assay) of hypoxic TECs in the presence or absence of PFCs. PFC-treated TECs showed an enhanced viability ($^{\#}P < 0.05$ vehicle versus PFCs). Data are expressed as average OD intensity ± 1 SD. (**B**) Time-course analysis of TEC apoptosis (TUNEL assay) induced by hypoxia in the presence or absence of PFCs. Hypoxia induced a significant increase of TECs apoptosis ($^{*}P < 0.05$ hypoxia versus normoxia) that was significantly reduced by PFCs ($^{\#}P < 0.05$ hypoxia + PFCs versus hypoxia). Data are expressed as the average number of green fluorescent apoptotic cells ± 1 SD in 10 different non-consecutive microscopic fields (×100 magnification). (**C**) Quantitative assessment of mitochondrial function (Mitotracker assay) after 24 h of hypoxia with or without PFCs ($^{*}P < 0.05$ hypoxia versus normoxia, $^{\#}P < 0.05$ hypoxia + PFCs versus hypoxia). Data are expressed as the average OD intensity ± 1 SD. (**D**) ATP production by TECs after 24 h of hypoxia with or without PFCs ($^{*}P < 0.05$ hypoxia versus normoxia, $^{\#}P < 0.05$ hypoxia + PFCs versus hypoxia). Data are expressed as average OD intensity ± 1 SD. Three experiments were performed with similar results.

Newman–Keuls, Kolmogorov–Smirnov or Dunnet multicomparison test as appropriate.

RESULTS

Protective effect of PFCs on hypoxia-induced TEC injury

When TECs were cultured for 168 h in hypoxic conditions, PFCs induced a significant increase of cell viability (XTT assay in Figure 2A). In addition, PFCs exerted an anti-apoptotic effect on hypoxic TECs at the different time points (TUNEL assay in Figure 2B). This protective effect of PFCs on hypoxia-induced TEC injury may be ascribed to the preservation of mitochondrial function (Mitotracker; Figure 2C) and of ATP production (Figure 2D). We then tested the effect of PFCs on the viability of TECs seeded within the inner surface of a polysulphone hollow fibre to mimic the operative condition of the RAD. We observed that PFCs significantly increased TEC viability (XTT assay in Figure 3A) and the release of the growth factors IGF-1, HGF and MSP (Figure 3B–D). Moreover, PFCs also increased ATP production in TECs seeded within the hollow fibre (Figure 3E).

PFCs inhibited apoptosis and functional alterations of TECs incubated with plasma collected from septic patients

Since we previously described that plasma from septic patients induced apoptosis of cultured TEC [6], we investigated the potential protective effect of PFCs in the same conditions. We selected 10 patients with sepsis-associated AKI and we collected plasma samples at the start of RRT. Clinical parameters of enrolled patients (including SOFA, APACHE II and RIFLE scores, need of mechanical ventilation and haemodynamic support, isolated microorganisms and antibiotic therapies) are detailed in Table 1. We first confirmed that septic plasma induced a significant increase of TEC apoptosis that was not observed with plasma from 10 healthy volunteers (Figure 4A) [6]. PFCs significantly decreased TEC apoptosis induced by septic plasma and the anti-apoptotic effect of PFCs was also observed in TECs cultured with LPS (30 ng/mL) or with inflammatory cytokines (TNF-α 20 ng/mL, IFN-γ 20 ng/mL) (Figure 4A). In addition, hypoxia sensitized TECs to the pro-apoptotic effect induced by septic plasma, LPS or inflammatory cytokines (Figure 4A). The

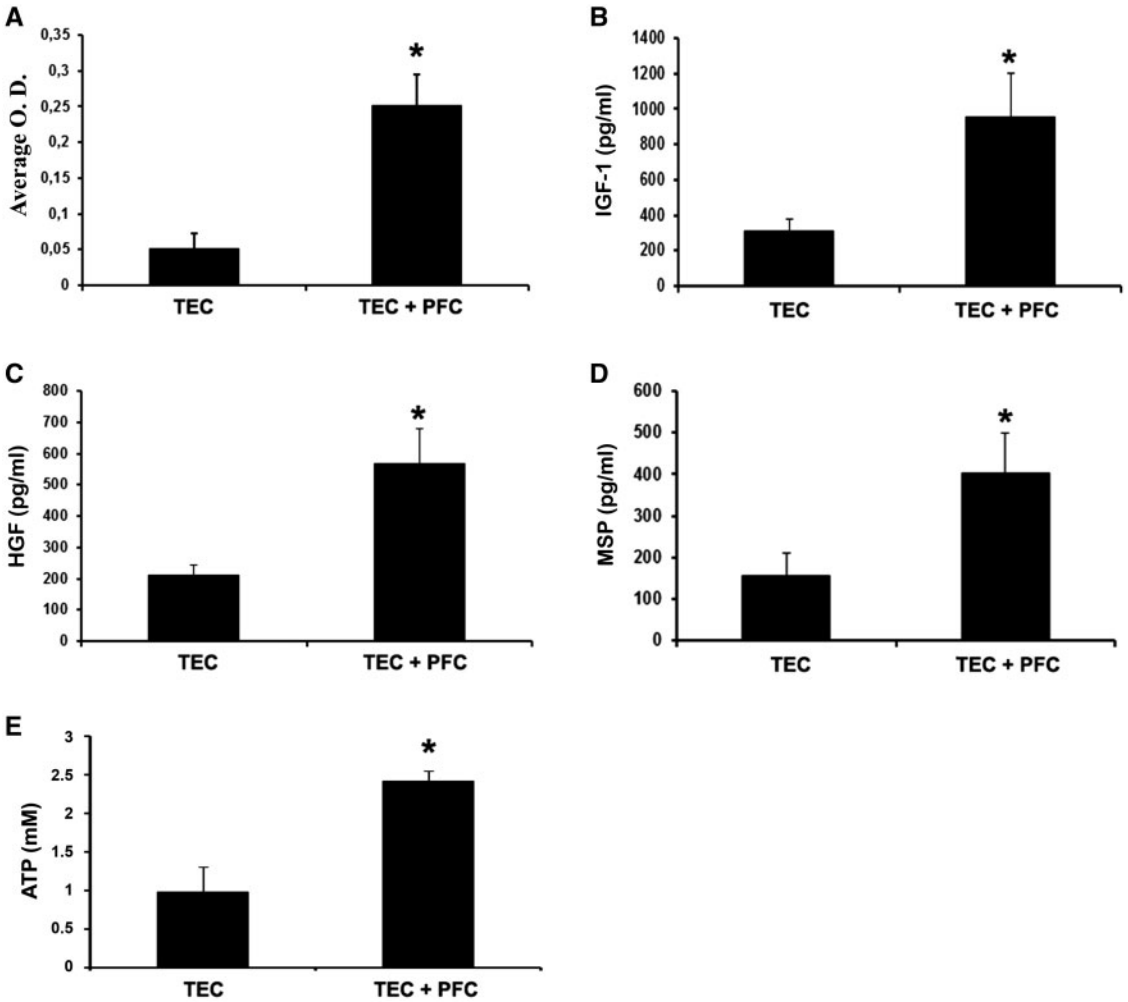

FIGURE 3: Biological effects of PFCs on TECs seeded within a polysulphone hollow fibre. (**A**) Analysis of the viability (XTT-based assay) of TECs seeded in a polysulphone hollow fibre in the presence or absence of PFCs. PFCs significantly increased cell viability (*P < 0.05 TECs versus TECs + PFCs). (**B–D**) ELISA determination of (**B**) IGF-1, (**C**) HGF or (**D**) MSP concentrations in supernatants of TECs cultured within the hollow fibre filter in the presence or absence of PFCs (*P < 0.05 TECs versus TECs + PFCs). (**E**) ATP production by TECs cultured within the hollow fibre filter in the presence or absence of PFCs (*P < 0.05 TECs versus TECs + PFCs). Five different experiments were performed with similar results.

Table 1. Main characteristics of the selected patients

ID	M/F	Age (years)	Origin of sepsis	Cause	AP II	SOFA	RIFLE	Amines	Nora (μ/kg/min)	Outcome	Antibiotics (after microbiology results)
1	F	72	Surgical wound	*Staphylococcus aureus*	28	13	F	Yes	0.6	Died	Vancomycin
2	M	67	Unknown	*Torulopsis glabrata* *Escherichia coli*	22	10	I	Yes	–	Survived	Amikacin, fluconazole
3	M	78	Urinary	*Klebsiella pneumoniae*	28	12	F	Yes	0.5	Survived	Gentamycin
4	M	71	Surgical wound	*Staphylococcus aureus*	29	15	F	Yes	0.9	Died	Teicoplanin
5	M	63	Lower airways	*Staphylococcus aureus*	30	11	I	Yes	0.8	Died	Vancomycin
6	M	72	Endocarditis	*Staphylococcus aureus*	21	7	I	Yes	0.5	Survived	Vancomycin
7	M	78	Lower airways	*Staphylococcus aureus*	22	6	R	Yes	–	Survived	Vancomycin
8	F	88	Lower airways	*Klebsiella pneumoniae*	24	11	F	Yes	0.7	Died	Tigecyclin
9	M	69	Bowel	*Escherichia coli*	30	15	F	Yes	0.6	Died	Amikacin
10	M	68	Bile duct	*Escherichia coli*	22	9	F	Yes	0.7	Survived	Amikacin

APII: APACHE II score; M: male; F: female; Nora: noradrenaline; R: risk; I: injury; F: failure.

observed enhancement of apoptosis was significantly decreased by PFCs (Figure 4A).

The detrimental effect of septic plasma on TECs was due to the activation of both mitochondrial- and death receptor-mediated pathways of apoptosis, as shown by ELISA for caspase-3, -8 and -9 activities (Figure 4B). Moreover, septic plasma significantly up-regulated the surface expression of the pro-apoptotic molecules Fas and Fas ligand in TECs (Figure 4C). In contrast, PFCs

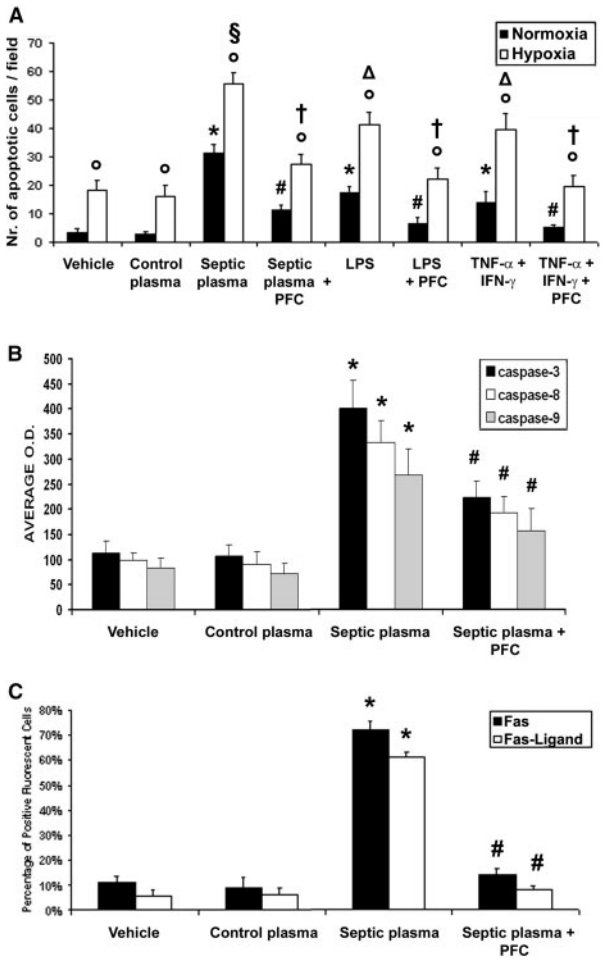

FIGURE 4: Anti-apoptotic effect induced by PFCs on TECs cultured with septic plasma and hypoxic condition. (**A**) Evaluation of TEC apoptosis (TUNEL assay) induced by septic plasma, LPS (30 ng/mL) or inflammatory cytokines (TNF-α 20 ng/mL, IFN-γ 20 ng/mL) in the presence or absence of PFCs. RPMI medium containing 10% septic plasma, LPS or inflammatory cytokines induced a marked increase of TEC apoptosis (black columns, *P < 0.05 septic plasma, LPS or cytokines versus vehicle). No pro-apoptotic effect was observed stimulating TECs with control healthy plasma. Co-incubation with PFCs significantly decreased the number of apoptotic cells (black columns, #P < 0.05 septic plasma versus septic plasma + PFCs, LPS versus LPS + PFCs, TNF-α + IFN-γ versus TNF-α + IFN-γ + PFCs). Hypoxia induced a significant increase in TEC apoptosis (°P < 0.05 hypoxia versus normoxia). In contrast, incubation with septic plasma but not with healthy plasma induced an enhanced effect on hypoxic-induced TEC apoptosis (white columns, §P < 0.05 septic plasma versus control plasma). This effect was significantly decreased by co-incubation with PFCs (white columns, †P < 0.05 septic plasma + PFCs versus septic plasma). Under hypoxia, incubation with LPS or inflammatory cytokines induced a marked increase of TEC apoptosis (white columns, ΔP < 0.05 LPS, TNF-α + IFN-γ versus vehicle). In the same manner, co-incubation with PFCs decreased the pro-apoptotic effect (white columns, †P < 0.05 LPS + PFCs versus LPS, TNF-α + IFN-γ versus TNF-α + IFN-γ + PFCs). Analysis of variance with Newmann–Keuls multicomparison test was performed. (**B**) Septic plasma induced a marked increase of caspase-3, -8 and -9 in TECs (*P < 0.05 septic plasma versus vehicle), whereas no significant increase in caspase-3, -8 and -9 activities was observed after

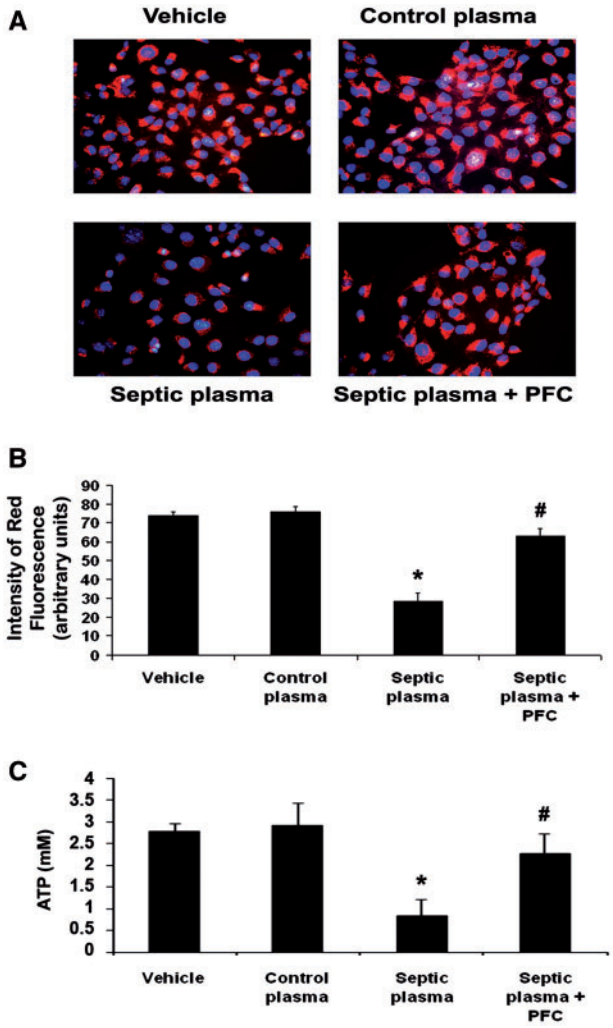

FIGURE 5: PFCs prevented septic plasma-mediated mitochondrial dysfunction in TECs. (**A**) The specific staining with the red fluorescent dye MitoTracker demonstrated that septic plasma but not control healthy plasma altered the mitochondrial membrane potential of TECs. PFCs preserved mitochondrial integrity in septic plasma-treated TECs. (**B**) Quantification of red fluorescence intensity of 10 different images from the same experiments. TECs incubated with septic plasma significantly decreased mitochondrial activity (*P < 0.05 septic plasma versus vehicle). PFCs prevented mitochondrial damage in septic plasma-treated TECs (#P < 0.05 septic plasma versus septic plasma + PFCs). (**C**) ATP production by TECs stimulated with plasma from septic patients in the presence or absence of PFCs (*P < 0.05 septic plasma versus vehicle, #P < 0.05 septic plasma versus septic plasma + PFCs).

FIGURE 4: Continued

stimulation with control plasma. Co-incubation of septic plasma with PFCs decreased caspase-3, -8 and -9 activities (#P < 0.05 septic plasma versus septic plasma + PFCs). (**C**) Septic plasma increased Fas and Fas ligand expression (*P < 0.05 septic plasma versus vehicle) in TECs. After stimulation with control plasma, no increase of Fas and Fas ligand expression was observed. PFCd significantly inhibited Fas and Fas ligand up-regulation (#P < 0.05 septic plasma versus septic plasma + PFCs).

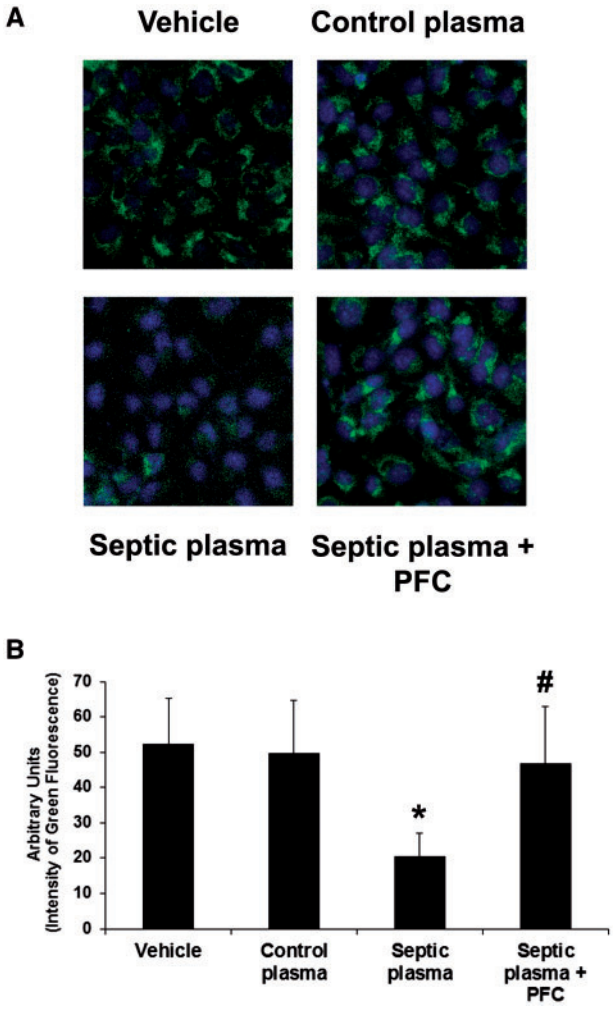

FIGURE 6: PFCs prevented a septic plasma–induced decrease of the mitochondrial protein PGC-1α. (**A**) Representative images showing that septic plasma but not control plasma decreased TEC expression of PGC-1α, a protein involved in mitochondrial biogenesis. PFCs exerted a protective effect in septic plasma–incubated TECs by preserving PGC-1α expression. (**B**) Quantification of fluorescence intensity in TECs (PGC-1α expression in 10 different non-consecutive microscopic fields). TECs incubated with septic plasma significantly decreased this marker of mitochondrial biogenesis (*P $<$ 0.05 septic plasma versus vehicle). PFCs prevented a PGC-1α decrease in septic plasma–treated TECs ($^{\#}$P $<$ 0.05 septic plasma versus septic plasma + PFCs).

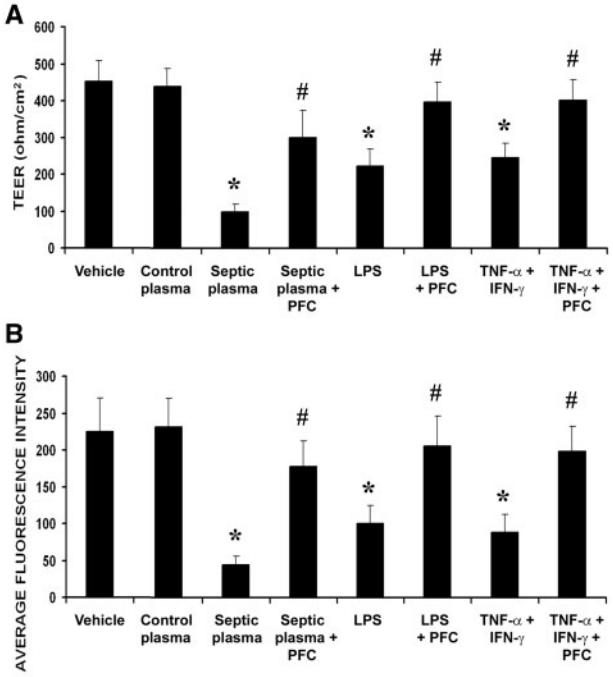

FIGURE 7: PFCs preserved cell polarity and albumin uptake in hypoxic TECs treated with septic plasma, LPS or inflammatory cytokines. (**A**) RPMI medium with 10% septic plasma, LPS (30 ng/mL) or inflammatory cytokines (TNF-α 20 ng/mL, IFN-γ 20 ng/mL) induced a marked alteration of TEC polarity assessed by a significant decrease of TEER (*P $<$ 0.05 septic plasma, LPS, TNF-α + IFN-γ versus vehicle). No significant alteration of TEER was observed stimulating TECs with control plasma. Co-incubation of PFCs with septic plasma, LPS or inflammatory cytokines prevented a loss of cell polarity ($^{\#}$P $<$ 0.05 septic plasma versus septic plasma + PFCs, LPS versus LPS + PFCs, TNF-α + IFN-γ versus TNF-α + IFN-γ + PFCs). (**B**) Quantitative analysis of three different FACS experiments showing that septic plasma (10% diluted in RPMI), LPS (30 ng/mL) or inflammatory cytokines (TNF-α 20 ng/mL, IFN-γ 20 ng/mL) significantly decreased albumin uptake by TECs (*P $<$ 0.05 septic plasma, LPS, TNF-α + IFN-γ versus vehicle). No alteration of albumin uptake was observed in TECs treated with control plasma. Co-incubation of PFCs with septic plasma, LPS or inflammatory cytokines limited the inhibition of albumin uptake ($^{\#}$P $<$ 0.05 septic plasma versus septic plasma + PFCs, LPS versus LPS + PFCs, TNF-α + IFN-γ versus TNF-α + IFN-γ + PFCs).

significantly reduced both caspase activation and Fas/Fas ligand expression (Figure 4B and C). The mitochondrial dysfunction induced by septic plasma in TECs was further confirmed by the specific staining with the red fluorescent dye MitoTracker: septic plasma but not control healthy plasma altered the mitochondrial membrane potential of TECs, whereas PFCs preserved mitochondrial integrity (Figure 5A and B). Furthermore, PFCs preserved ATP production (Figure 5C) and the expression of PGC-1α, a protein involved in mitochondrial biogenesis that was significantly reduced in TECs exposed to septic plasma (Figure 6A and B).

In addition to the pro-apoptotic effect, septic plasma, LPS and inflammatory cytokines induced functional alterations of TECs, such as the loss of cell polarity assessed by TEER (Figure 7A) and the ability to uptake albumin (Figure 7B). The co-incubation with PFCs preserved cell polarity and albumin uptake of TECs cultured in different detrimental conditions (Figure 7A and B). In accordance with the loss of cell polarity, septic plasma induced down-regulation of the tight junction protein ZO-1 and the endocytic receptor megalin (Figure 8A and B). The decreased expression of ZO-1 and megalin in septic plasma–incubated TECs was less marked when cells were cultured in the presence of PFCs (Figure 8A and B).

PFCs promoted CD133^{+} renal progenitor cell proliferation and differentiation towards a tubular-like phenotype

Since a percentage of cells present in the RAD may have a renal progenitor phenotype [8], we analysed the effect of PFCs on cultured CD133^{+} human renal progenitor proliferation and

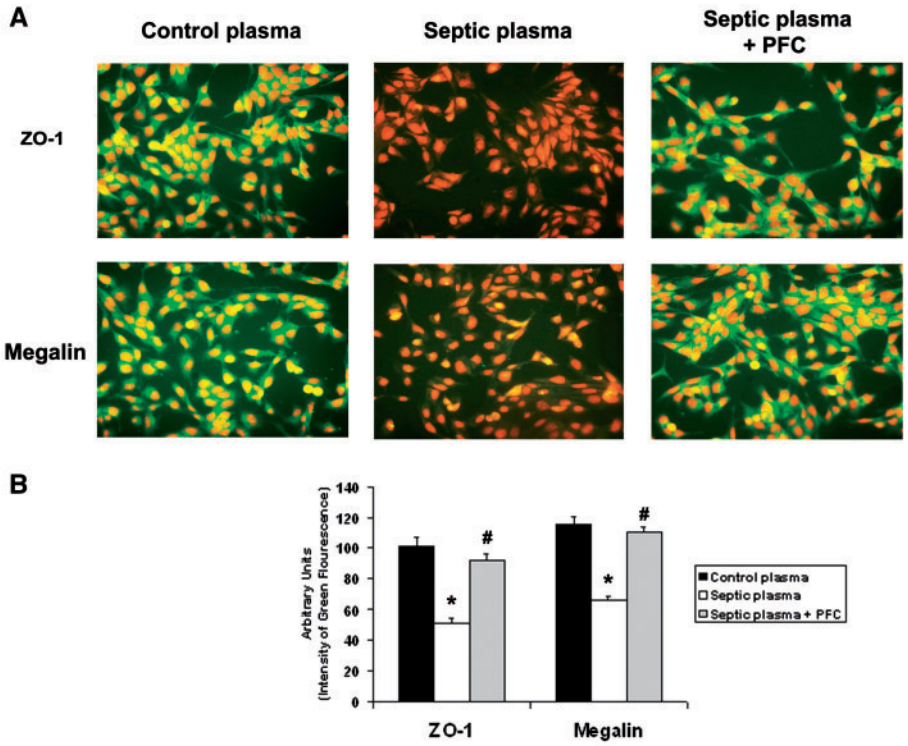

FIGURE 8: PFCs prevented septic plasma–induced down-regulation of the tight junction protein ZO-1 and the endocytic receptor megalin in TECd. (**A**) Representative images showing that septic plasma, but not control plasma, decreased expression of ZO-1 and megalin in TECd. PFCd preserved the expression of these epithelial/tubular markers in septic plasma–stimulated TECs. (**B**) Quantification of green fluorescence intensity of 10 different non-consecutive microscopic fields: septic plasma significantly decreased ZO-1 and megalin expression in TECs (white columns, $^{*}P < 0.05$ septic plasma versus control plasma). PFCs prevented the loss of expression of ZO-1 and megalin in septic plasma-stimulated TECs (grey columns, $^{\#}P < 0.05$ septic plasma versus septic plasma + PFCs).

differentiation. We found that PFCs induced a slight proliferative effect on $CD133^{+}$ renal progenitors (BrdU assay in Figure 9A). This effect was similar to that observed in the presence of 10 ng/mL of epidermal growth factor (EGF) and 10 ng/mL of platelet-derived growth factor-α (PDGF-α), molecules known to mediate progenitor cell proliferation [27, 28]. Moreover, PFCs induced the differentiation of $CD133^{+}$ renal progenitors towards an epithelial/tubular-like phenotype, as shown by the acquisition of cell polarity assessed by the increase of TEER (Figure 9B) and by the induction of scattering, branching morphogenesis and formation of tubular-like structures *in vitro* when cells were cultured onto Matrigel-coated plates (Figure 10A and B). This effect was similar to that observed when culturing cells in a medium containing fibroblast growth factor-4 (10 ng/mL) and hepatocyte growth factor (20 ng/mL), which are known to induce progenitor cell differentiation (Figure 10A and B) [29]. Furthermore, PFCs induced in $CD133^{+}$ renal progenitors the expression of markers of mature TECs such as E-cadherin, megalin, alkaline phosphatase, aminopeptidase A, aquaporin- 1 (AQP-1) and lipocalin-2 (NGAL) (Figure 11A and B).

DISCUSSION

In the present study, we demonstrated that the O_2 carrier of PFCs preserves TEC viability under hypoxic conditions. This effect was observed both *in vitro*, by using collagen-coated transwell plates in the presence of low O_2 tension, and *ex vivo*, by re-creating an extracorporeal circuit in which TECs were seeded within the hollow fibres of a polysulphone hemofilter. A similar beneficial effect was observed for TEC injury induced by septic plasma: cells were protected from apoptosis, loss of cell polarity and dedifferentiation. We also found that PFCs promoted proliferation and differentiation of $CD133^{+}$ human renal progenitors towards an epithelial/tubular-like phenotype. This finding suggests that high O_2 tension has a putative role in renal progenitor cell differentiation, thus favoring tissue regeneration. Taken together, these results advocate for the potential use of PFCs to improve the performance of RADs for the treatment of sepsis-associated AKI and multiple organ failure.

The association between AKI and sepsis leads to an increase in mortality and progression towards chronic kidney disease [30]. In recent years, several studies have questioned the old pathogenic model of sepsis-induced AKI based only on systemic hypotension and tissue hypoperfusion. In contrast, the role of detrimental circulating factors (e.g. bacterial products, cytokines, molecules released after cell lysis, etc.) is now evident [3, 4, 31]. We previously demonstrated that plasma collected from critically ill patients with sepsis-associated AKI induces apoptosis and functional alterations of glomerular and tubular epithelial cells [24]. This is in accordance with the results recently published by Lerolle *et al.* [32] showing the presence of kidney tubular cell apoptosis and leucocyte infiltration in early postmortem autopsy of patients with sepsis and AKI. A primary mechanism of TEC dysfunction is mitochondrial injury leading

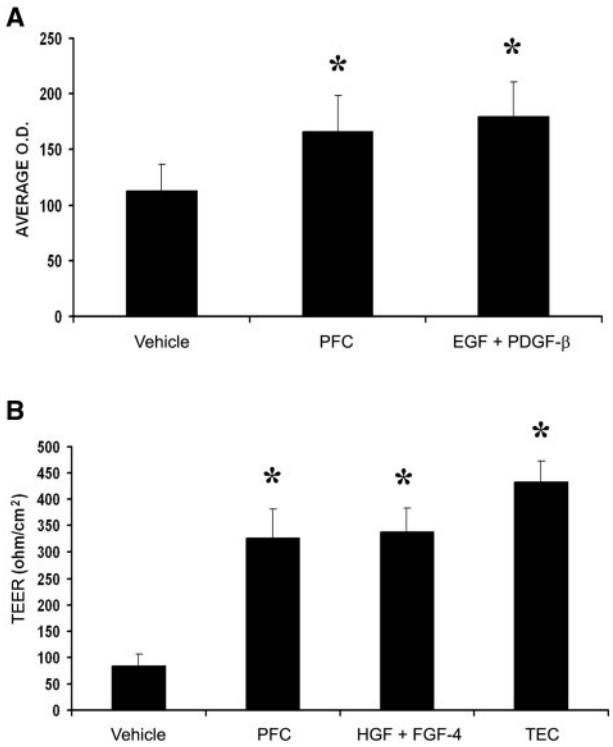

FIGURE 9: PFCs induced proliferation and differentiation of human CD133$^+$ renal progenitors. (**A**) PFCs increased the proliferation rate of CD133$^+$ renal progenitors evaluated by the XTT-based assay. Similar results were obtained using a cell culture medium containing 10 ng/mL EGF and 10 ng/mL PDGF-α (*P $<$ 0.05 PFCs or EGF + PDGF-α versus vehicle). (**B**) As assessed by TEER analysis, PFCs induced differentiation of CD133$^+$ renal progenitors. Similar results were obtained using a cell culture medium containing 10 ng/mL fibroblast growth factor-4 and 20 ng/mL hepatocyte growth factor (*P $<$ 0.05 PFCs or HGF + fibroblast growth factor 4 versus vehicle). Unstimulated TECs were used as an experimental control (*P $<$ 0.05 TECs versus vehicle).

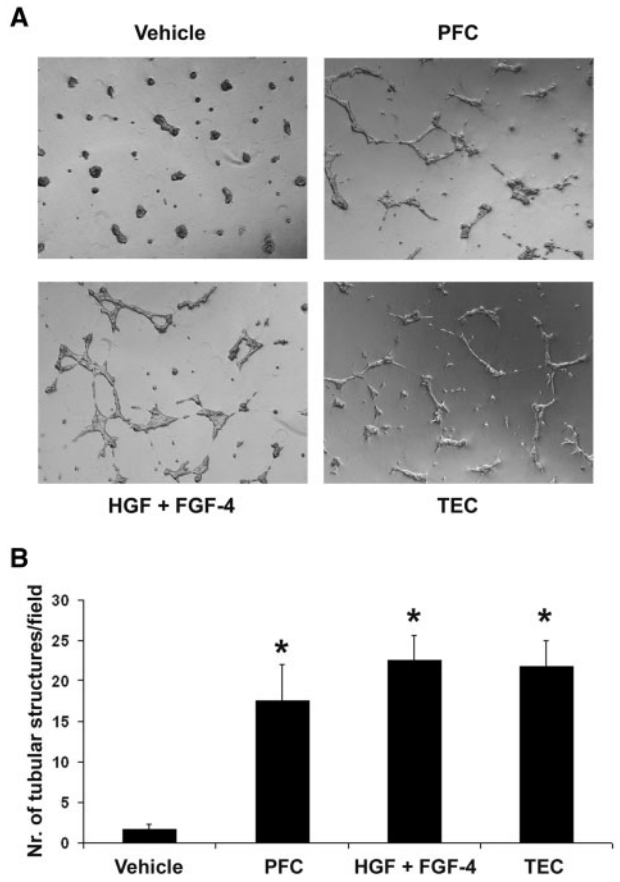

FIGURE 10: PFCs induced scattering, branching morphogenesis and formation of tubular-like structures of CD133$^+$ renal progenitors. (**A**) Representative images of CD133$^+$ renal progenitors cultivated in the presence of PFCs for 48 h on Matrigel-coated plates showing the formation of tubular-like structures. Similar results were obtained using a cell culture medium containing 10 ng/mL fibroblast growth factor 4 (FGF-4) and 20 ng/mL hepatocyte growth factor. Unstimulated TECs were used as an experimental control. Magnification: ×100. Five different experiments were performed with similar results. (**B**) Quantification of the number of tubular-like structures in 10 different non-consecutive fields. PFCs induced a significant increase of tubular-like structures formed by CD133$^+$ renal progenitors cultured on Matrigel. Similar results were observed in the presence of a cell culture medium containing FGF-4 (10 ng/mL) and HGF (20 ng/mL) (*P $<$ 0.05 PFCs or HGF + FGF-4 versus vehicle). Unstimulated TECs were used as an experimental control (*P $<$ 0.05 TECs versus vehicle).

to organelle autodigestion (mitophagy) or disruption, thus causing caspase release and cell apoptosis [33]. Consistently, different authors have demonstrated that the sepsis milieu halts renal oxidative metabolism independently from arterial oxygen tension [4, 33]. This phenomenon seems related to cytochrome dysfunction with local production of oxidizing species [34].

We herein show that PFCs significantly inhibited septic plasma-induced TEC apoptosis. The anti-apoptotic effect of PFCs was ascribed to the limitation of both the death receptor and mitochondrial pathway of apoptosis as demonstrated by the decrease of Fas/Fas ligand expression and of caspase-3, -8 and -9 activities and by the maintenance of potential mitochondrial membrane and of PGC-1α expression. These findings have a particular relevance considering the new unifying pathogenic theories, suggesting that sepsis-induced AKI is associated with an adaptive response of tubular cells to injury orchestrated by mitochondria and by bioenergetic modulation [35]. Moreover, Tran *et al.* [36] recently demonstrated that PGC-1α knockout mice had normal renal function but suffered persistent injury following endotoxemia, suggesting that the up-regulation of PGC-1α is essential for recovery from AKI.

Another typical tubular cell alteration in the course of sepsis-associated AKI is the loss of cell polarity with the concomitant down-regulation of sodium, urea and glucose transporters and of tight junction molecules such as ZO-1 [37–39]. These functional alterations induced by the inflammatory microenvironment lead to an increased delivery of sodium and chloride to the distal tubule segment, thus triggering tubuloglomerular feedback and consequently GFR impairment [40]. In this study, we found that PFCs preserved TEC polarity and function as demonstrated by the maintenance of TEER, ability to uptake albumin and megalin/ZO-1 expression.

Considering the putative role of circulating mediators in sepsis-associated AKI and multiple organ failures, different

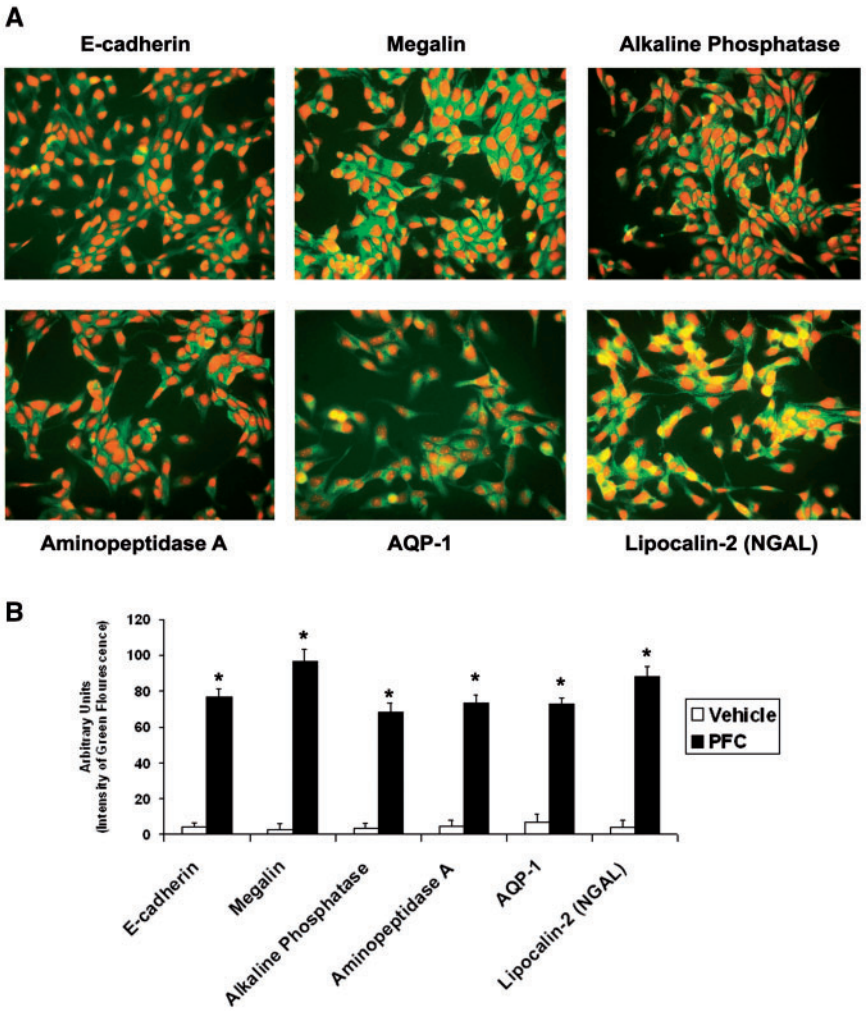

FIGURE 11: PFCs induced the expression of epithelial/tubular markers in CD133$^+$ renal progenitor cells. Representative (**A**) fluorescence microscope images and (**B**) relative quantification of fluorescence intensity in 10 non-consecutive microscopic fields of CD133$^+$ renal progenitors cultured in the presence of PFCs for 48 h and stained for the following epithelial/tubular markers: E-cadherin, megalin, alkaline phosphatase, aminopeptidase A, aquaporin-1 (AQP-1) and lipocalin-2 (NGAL). PFCs significantly increased the expression of E-cadherin, megalin, alkaline phosphatase, aminopeptidase A, AQP-1 and NGAL (*$P < 0.05$ PFCs versus vehicle).

extracorporeal blood purification techniques have been developed to control metabolic, electrolyte and acid–base alterations as well as to remove from the bloodstream harmful inflammatory mediators [41, 42]. Recent randomized clinical trials did not show a beneficial effect induced by high convective dialysis dose on mortality of septic AKI patients [4, 43, 44]. Several preclinical and clinical ongoing studies are investigating the possible role of high cut-off or adsorption membranes on mortality and AKI progression in septic patients [45]. In this setting, the development of cell-based therapies able to support standard diffusion- and convection-based extracorporeal techniques may improve the suboptimal treatment of sepsis-associated AKI.

A bioartificial RAD containing living TECs has been developed that demonstrates adsorptive, metabolic and endocrine functions of the normal kidney [10]. The use of RAD ameliorated Gram-negative bacteria–induced septic shock in uremic animals [10]; moreover, in phase I/II clinical trials, RAD induced a significant decrease in 28-day mortality and a more rapid recovery of renal function in patients with sepsis-associated AKI [8, 10, 11]. On this basis, the association of RAD with conventional haemofiltration therapy may improve the outcome of these critically ill patients. However, the widespread clinical use of RAD is limited by major problems, such as the preservation of long-term TEC viability in large-scale culture and the limited supply of nutrients and O_2 within hollow fibres [46].

PFC molecules are able to solubilize oxygen and other gasses in aqueous solutions; this effect is mainly dependent on gas partial pressure in accordance to the Henry's law [25]. Of note, as PFC molecules are highly hydrophilic, they remain compartmentalized in the extracellular space and do not have a direct cytoprotective role [13]. The use of PFCs as O_2 carriers began as early as the 1960s [47] and later PFC emulsions were used to enhance O_2 transport in cell cultures [10]. In engineered cardiac tissues, PFCs have been shown to improve mass transport of O_2 by increasing the convective and diffusive flux [12]. Moreover, in another study aimed at investigating O_2 transport in tubular vessels, a mathematical model demonstrated that supplementation of PFCs generally increased the O_2 transfer rate for both

dispersion patterns, particularly at high O_2 tension [12, 46, 47]. These observations acquire a particular relevance for the clinical application of bioreactors to support organ failure, where living cells may suffer from hypoxia and lack of nutrients. This is in accordance with previously published papers showing that PFCs improve the metabolic function of hepatocytes seeded in a radial-flow bioreactor [12, 14]. In this study we observed that PFCs improved TEC viability as assessed by preservation of mitochondrial activity and biogenesis. This effect could be a consequence of the enhanced oxygen delivery to mitochondria combined with the increased local clearance of ROS and other detrimental/oxidizing molecules as NO derivatives [48]. Our results suggest that the maintenance of TEC viability may improve the clearance of circulating inflammatory and immunologically active mediators, even in hypoxic conditions. This is of specific relevance in the setting of RAD, where TECs seeded in the inner part of the hollow fibre are concomitantly exposed to low O_2 tension and to the detrimental activities of pro-apoptotic molecules circulating in the plasma of patients with sepsis-associated AKI. Moreover, the maintenance of viable TECs within the RAD may also lead to the release of tubulotrophic growth factors such as IGF-1, HGF and MSP that potentially contribute to tissue regeneration after AKI [26].

Another interesting finding of this study is the potential use of PFCs for renal progenitor cell differentiation. Indeed, PFCs are employed as preservation liquid in islet transplantation as well as for their ability to improve β-cell viability and to induce the differentiation of pancreatic precursors into β-cells in the presence of high O_2 tension [12]. We herein found that PFCs induced differentiation of $CD133^+$ human renal progenitor cells towards a tubular phenotype, as assessed by the acquisition of cell polarity typical of mature epithelial cells, by the ability to induce scattering and branching morphogenesis and by the increased expression of tubular-specific molecules such as megalin, alkaline phosphatase, aminopeptidase A, AQP-1 and NGAL. This may have a peculiar relevance for the clinical application of RAD, in which the presence of kidney-derived cells with a progenitor phenotype has been recently demonstrated [8, 11].

We have to acknowledge that this study is providing only *ex vivo* and *in vitro* results: indeed, this is a preliminary experience that could lead to future development of a RAD with enhanced metabolic activities, similar to what was recently published about the bioreactor using viable hepatocytes [14]. However, we believe that our results may open some new clinical scenarios. To date, the major limits to bioreactor use are the difficulties related to storage and transport; interestingly, preservation medium containing PFCs may address both these issues. Moreover, different PFC molecules have been approved for intravenous use during cardiac stenting, for ocular surgery and as contrast agents during radiology procedures [15, 49, 50]. Hence, one could hypothesize a future use of PFC-based solutions during extracorporeal therapies for organ support in critically ill patients. Additionally, the RAD architecture should minimize PFC diffusion to patients' blood, as fluorocarbon molecules should not pass the tubular cell layer.

In conclusion, the results of the present study suggest that PFCs protect TECs from injury related to hypoxia and inflammatory mediators and promotes $CD133^+$ renal progenitor cell differentiation. These observations have a potential relevance for improving the metabolic performances of bioartificial RAD for the therapeutic treatment of sepsis-associated AKI and multiple organ failure.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Informed consent was obtained according to the Declaration of Helsinki. The Internal Review Board of the San Bortolo Hospital in Vicenza authorized this study. Written informed consent was obtained from patients for publication of their individual details and accompanying images in this article. The consent form is held by the authors and is available for review by the editor-in-chief.

AVAILABILITY OF DATA AND MATERIAL

All data and material are fully available (contact V.C.).

AUTHORS' CONTRIBUTIONS

V.C. conceived the experiments, wrote the article and analysed and interpreted data. D.M. performed the experiments, wrote the article and analysed data. A.D.Q. performed the experiments and analysed data. S.D. wrote the article and analysed data. F.F. performed the experiments. G.M.V. performed the experiments and contributed reagents/materials/analysis tools. A.B. contributed to the study design and data analysis. M.Q. wrote the article. M.M. contributed reagents/materials/analysis tools. C.O. collected patient data. M.S. performed some experiments and contributed reagents/materials/analysis. F.G. contributed reagents/materials/analysis tools. F.D.C. contributed to the study design and data analysis. G.Cas. conceived the experiments. L.G. conceived the experiments, reviewed the article and analysed and interpreted data. G.Cam. conceived the experiments, reviewed the article and analysed and interpreted data. C.R. conceived the experiments, wrote the article and analysed and interpreted data. All authors read and approved the final article.

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CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

REFERENCES

1. Chertow GM, Burdick E, Honour M *et al.* Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16: 3365–3370
2. Bagshaw SM, Uchino S, Bellomo R *et al.* Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clin J Am Soc Nephrol* 2007; 2: 431–439

3. Cantaluppi V, Quercia AD, Dellepiane S *et al*. [New mechanisms and recent insights in the pathogenesis of acute kidney injury (AKI)]. *G Ital Nefrol* 2012; 29: 535–547
4. Dellepiane S, Marengo M, Cantaluppi V. Detrimental cross-talk between sepsis and acute kidney injury: new pathogenic mechanisms, early biomarkers and targeted therapies. *Crit Care* 2016; 20: 61
5. Romanovsky A, Morgan C, Bagshaw SM. Pathophysiology and management of septic acute kidney injury. *Pediatr Nephrol* 2014; 29: 1–12
6. Mariano F, Cantaluppi V, Stella M *et al*. Circulating plasma factors induce tubular and glomerular alterations in septic burns patients. *Crit Care* 2008; 12: R42
7. Peng Z, Singbartl K, Simon P *et al*. Blood purification in sepsis: a new paradigm. *Contrib Nephrol* 2010; 165: 322–328
8. Pino CJ, Yevzlin AS, Tumlin J *et al*. Cell-based strategies for the treatment of kidney dysfunction: a review. *Blood Purif* 2012; 34: 117–123
9. Bussolati B, Camussi G. Adult stem cells and renal repair. *J Nephrol* 2006; 19: 706–709
10. Song JH, Humes HD. Renal cell therapy and beyond. *Semin Dial* 2009; 22: 603–609
11. Song JH, Humes HD. The bioartificial kidney in the treatment of acute kidney injury. *Curr Drug Targets* 2009; 10: 1227–1234
12. Hosgood SA, Nicholson ML. The role of perfluorocarbon in organ preservation. *Transplantation* 2010; 89: 1169–1175
13. Cabrales P, Intaglietta M. Blood substitutes: evolution from non-carrying to oxygen and gas carrying fluids. *J Am Soc Artif Intern Organs* 2013; 59: 337–354
14. Nieuwoudt MJ, Moolman SF, Van Wyk KJ *et al*. Hepatocyte function in a radial-flow bioreactor using a perfluorocarbon oxygen carrier. *Artif Organs* 2005; 29: 915–918
15. Wall TC, Califf RM, Blankenship J *et al*. Intravenous Fluosol in the treatment of acute myocardial infarction. Results of the Thrombolysis and Angioplasty in Myocardial Infarction 9 Trial. TAMI 9 Research Group. *Circulation* 1994; 90: 114–120
16. Riess JG. Perfluorocarbon-based oxygen delivery. *Artif Cells Blood Substit Immobil Biotechnol* 2006; 34: 567–580
17. Bando K, Teramoto S, Tago M *et al*. Oxygenated perfluorocarbon, recombinant human superoxide dismutase, and catalase ameliorate free radical induced myocardial injury during heart preservation and transplantation. *J Thorac Cardiovasc Surg* 1988; 96: 930–938.
18. Singer M, Deutschman CS, Seymour CW *et al*. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 801–810
19. Van Biesen W, Vanholder R, Lameire N. Defining acute renal failure: RIFLE and beyond. *Clin J Am Soc Nephrol* 2006; 1: 1314–1319
20. Bussolati B, Bruno S, Grange C *et al*. Isolation of renal progenitor cells from adult human kidney. *Am J Pathol* 2005; 166: 545–555
21. Reznik ON, Bagnenko SF, Loginov IV *et al*. The use of oxygenated perfluorocarbonic emulsion for initial in situ kidney perfusion. *Transplant Proc* 2008; 40: 1027–1028
22. Cantaluppi V, Dellepiane S, Tamagnone M *et al*. Neutrophil gelatinase associated lipocalin is an early and accurate biomarker of graft function and tissue regeneration in kidney transplantation from extended criteria donors. *PLoS One* 2015; 10: e0129279
23. Humes HD, MacKay SM, Funke AJ *et al*. Tissue engineering of a bioartificial renal tubule assist device: in vitro transport and metabolic characteristics. *Kidney Int* 1999; 55: 2502–2514
24. Cantaluppi V, Weber V, Lauritano C *et al*. Protective effect of resin adsorption on septic plasma-induced tubular injury. *Crit Care* 2010; 14: R4
25. Arab A, Kuemmerer K, Wang J *et al*. Oxygenated perfluorochemicals improve cell survival during reoxygenation by pacifying mitochondrial activity. *J Pharmacol Exp Ther* 2008; 325: 417–424
26. Cantaluppi V, Biancone L, Romanazzi GM *et al*. Macrophage stimulating protein may promote tubular regeneration after acute injury. *J Am Soc Nephrol* 2008; 19: 1904–1918
27. Floege J, Burns MW, Alpers CE *et al*. Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int* 1992; 41: 297–309
28. Kato Y, Tapping RI, Huang S *et al*. Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* 1998; 395: 713–716
29. Flaquer M, Romagnani P, Cruzado JM. Growth factors and renal regeneration. *Nefrologia* 2010; 30: 385–393
30. Schrier RW, Wang W. Acute renal failure and sepsis. *N Engl J Med* 2004; 351: 159–169
31. Boomer JS, To K, Chang KC *et al*. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* 2011; 306: 2594–2605
32. Lerolle N, Nochy D, Guérot E *et al*. Histopathology of septic shock induced acute kidney injury: apoptosis and leukocytic infiltration. *Intensive Care Med* 2010; 36: 471–478
33. Cantaluppi V, Quercia AD, Dellepiane S *et al*. Interaction between systemic inflammation and renal tubular epithelial cells. *Nephrol Dial Transplant* 2014; 29: 2004–2011
34. Quoilin C, Mouithys-Mickalad A, Lécart S *et al*. Evidence of oxidative stress and mitochondrial respiratory chain dysfunction in an in vitro model of sepsis-induced kidney injury. *Biochim Biophys Acta* 2014; 1837: 1790–1800
35. Emlet DR, Shaw AD, Kellum JA. Sepsis-associated AKI: epithelial cell dysfunction. *Semin Nephrol* 2015; 35: 85–95
36. Tran M, Tam D, Bardia A *et al*. PGC-1α promotes recovery after acute kidney injury during systemic inflammation in mice. *J Clin Invest* 2011; 121: 4003–4014
37. Zegers MMP, O'Brien LE, Yu W *et al*. Epithelial polarity and tubulogenesis in vitro. *Trends Cell Biol* 2003; 13: 169–176
38. Morrell ED, Kellum JA, Hallows KR *et al*. Epithelial transport during septic acute kidney injury. *Nephrol Dial Transplant* 2014; 29: 1312–1319
39. Lee S-Y, Shin J-A, Kwon HM *et al*. Renal ischemia-reperfusion injury causes intercalated cell-specific disruption of occludin in the collecting duct. *Histochem Cell Biol* 2011; 136: 637–647
40. Gomez H, Ince C, De Backer D *et al*. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. *Shock* 2014; 41: 3–11
41. Marengo M, Dellepiane S, Cantaluppi V. Extracorporeal treatments in patients with acute kidney injury and sepsis. *Contrib Nephrol* 2017; 190: 1–18
42. Dellepiane S, Medica D, Quercia AD *et al*. The exciting 'bench to bedside' journey of cell therapies for acute kidney injury and renal transplantation. *J Nephrol* 2017; 30: 319–336
43. Joannes-Boyau O, Honoré PM, Perez P *et al*. High-volume versus standard-volume haemofiltration for septic shock patients with acute kidney injury (IVOIRE study): a multicentre randomized controlled trial. *Intensive Care Med* 2013; 39: 1535–1546
44. Ronco C, Inguaggiato P, D'intini V *et al*. The role of extracorporeal therapies in sepsis. *J Nephrol* 2003; 16(Suppl 7): S34–S41
45. Honore PM, Jacobs R, Joannes-Boyau O *et al*. Septic AKI in ICU patients. diagnosis, pathophysiology, and treatment type, dosing, and timing: a comprehensive review of recent and future developments. *Ann Intensive Care* 2011; 1: 32
46. Saito A, Sawada K, Fujimura S *et al*. Evaluation of bioartificial renal tubule device prepared with lifespan-extended human renal proximal tubular epithelial cells. *Nephrol Dial Transplant* 2012; 27: 3091–3099
47. Henkel-Honke T, Oleck M. Artificial oxygen carriers: a current review. *AANA J* 2007; 75: 205–211
48. Pollack GL, Kennan RP, Holm GT. Solubility of inert gases in PFC blood substitute, blood plasma, and mixtures. *Biomater Artif Cells Immobil Biotechnol* 1992; 20: 1101–1104
49. Rejdak R, Choragiewicz T, Moneta-Wielgos J *et al*. Intraoperative macula protection by perfluorocarbon liquid for the metallic intraocular foreign body removal during 23-gauge vitrectomy. *J Ophthalmol* 2017; 2017: 6232151
50. Li DS, Yoon SJ, Pelivanov I *et al*. Polypyrrole-coated perfluorocarbon nanoemulsions as a sono-photoacoustic contrast agent. *Nano Lett* 2017; 17: 6184–6194

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