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Application of Human Plasma/Serum to Cell Culture In Vitro: A Translational Research Approach to Better Define Disease Mechanisms

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ABSTRACT

In vitro cell culture experiments play an important role in medical research. Various cellular mechanisms and signaling pathways have been identified with in vitro experimental techniques. Unfortunately, the clinical and translational impact of these studies is often limited due to their inability to closely resemble physiological or pathophysiological milieus in cell culture and the use of unrealistic experimental conditions. Thus, further developments must be made to improve the translation of in vitro cell culture work. The application of human plasma or serum as a stimulus for cells, human or otherwise, is a relatively new approach that ultimately overcomes many of the in vitro limitations and provides a more physiologically relevant model. While this technique has been used for the investigation of various diseases and pharmacological mechanisms, discrepancies remain regarding the appropriate methodologies. This review provides insight into recent findings through the application of human plasma or serum as stimuli, as well as an analysis of methodological considerations and suggestions for future directions.

Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; A549, human lung adenocarcinoma cells; AEC, primary human alveolar epithelial cells; Akt, protein kinase B; Ang2, angiopoietin-2; BBB, blood–brain barrier; Bcl-2, B-cell lymphoma 2; BLEC, brain-like endothelial cells; C2C12, myoblasts; CCL-5, C-C motif chemokine ligand 5; CD-3, cluster of differentiation 3; CEM, human T lymphoid cell line; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CTLA4, cytotoxic T-lymphocyte–associated protein 4; CXCL13, chemokine ligand 13; DKA, diabetic ketoacidosis; EDTA, ethylenediaminetetraacetic acid; eNOS, endothelial nitric oxide synthase; Erk1/2, extracellular signal–regulated kinase 1/2; FABP4, fatty acid–binding protein-4; FBS, fetal bovine serum; FCS, fetal calf serum; FLRG, follistatin-related gene; FTH1, ferritin heavy chain 1; G-CSF, granulocyte colony stimulating factor; GLUT4, glucose transporter type 4; GPX4, glutathione peroxidase 4; HAE, human airway epithelial cells; HCAEC, human coronary artery endothelial cells; HCMEC, human cerebral microvascular endothelial cells; hCSC, human cardiac stem cells; Hep-2, human epithelial cells; HGF, hepatocyte growth factor; hMSC, human mesenchymal stem cells; HMVEC, human microvascular endothelial cells; hOB, human osteoblasts; HPAAF, human pulmonary arterial adventitial fibroblasts; HPC, human hippocampal progenitor cells; HPMVEC, human pulmonary microvascular endothelial cells; Hsp27, heat shock protein 27; HSPCs, hematopoietic stem and progenitor cells; HTO, 3D human testicular organoids; Huh7.5, human hepatocellular carcinoma cell line; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; IFNγ, interferon gamma; IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-7, interleukin 7; IL-8, interleukin 8; IP-10, interferon gamma-induced protein 10; IU/mL, international units per milliliter; K562, human cell line derivative of chronic myelogenous leukemia; L6-GLUT4myc, rat myoblast cell line; LC–MS/MS, liquid chromatography with tandem mass spectrometry; LHCN-M2, immortalized human skeletal myoblasts; LNCaP, androgen-sensitive human prostate adenocarcinoma cells; LoVo, human colon cancer cell line; LPS, lipopolysaccharides; MAFbx, atrogin-1; MCP-1, monocyte chemotactic protein-1; MCVEC, mouse cerebrovascular endothelial cells; MH-S, murine alveolar macrophage cell line; MIP1B, macrophage inflammatory protein 1 beta; MIS-C, multisystem inflammatory syndrome in children; MMP-9, matrix metalloproteinase 9; MRC-5, human lung fibroblast cell line; mTOR, mammalian target of rapamycin; MuRF-1, muscle RING-finger protein 1; NFkB, nuclear factor kappa B; NMR, nuclear magnetic resonance; NO, nitric oxide; NOX2, NADPH oxidase 2; Nuli-1, human airway epithelial cell line; p38MAPK, p38 mitogen-activated protein kinase; P53, tumor protein P53; P62, sequestosome 1; P70 S6K, ribosomal protein S6 kinase B1; PBMCs, human peripheral blood mononuclear cells; PC-3, human prostate cancer cell line; PHH, primary human hepatocytes; PPARγ, peroxisome proliferator-activated receptor gamma; Raji, human B lymphoid cell line; ROS, reactive oxygen species; S100A, S100 calcium-binding protein A; SAT1, spermidine; SHBG, sex hormone–binding globulin; SLC7A11, solute carrier family 7 member 11; SREBP-1c, sterol regulatory element binding protein-1c; STAT3, signal transducer and activator of transcription 3; STC, mixed seminiferous tubule cells; Tie2, angiopoietin-1 receptor; TIG-1, human fetal lung fibroblasts; TIG-3S, human fetal skin fibroblasts; TNF-α, tumor necrosis factor alpha; TREM-1, triggering receptor expressed on myeloid cells 1; v/v, volume/volume; VEGF, vascular endothelial growth factor; γ-H2AX, histone family member.

1 | Introduction

Much of our current knowledge regarding biological mechanisms stems from in vitro investigations. Cell culture has proven to be a feasible and flexible method that allows for the comprehensive investigation of cellular physiology and disease mechanisms, as well as the efficacy and toxicity of drugs [1–3]. The ability of in vitro cell culture experiments to provide clinically meaningful data, however, is based on the assumption that the in vitro conditions provide an accurate representation of in vivo milieu/responses [1]. Unfortunately, traditional in vitro systems often utilize culture media conditions and simplified stimuli to optimize replicability [1, 4]. For example, lipopolysaccharides (LPS) or individual cytokines are commonly used stimuli, with the goal of exposing cells in culture to factors identified as key pathological components [5, 6]. However, these simplified treatments are devoid of many of the components present in human blood and thus are unable to replicate the complexity of a physiological scenario [1, 4, 7]. Additionally, culture media typically contain excessive growth factors and nutrients designed to promote cell proliferation, thereby constraining the reliability of in vitro results [8].

To develop a translational research approach, it is important to evaluate effective methods to investigate pathological and pharmacological properties in cultured cells. Although in vivo experiments are considered the gold standard approach for physiological studies, they are limited by the reduced availability of appropriate model systems (i.e., animals), ethical considerations, and the inherent complexity associated with utilizing preclinical animal models [4]. Thus, there is an ongoing need for a physiologically representative in vitro model capable of improving the translation of research findings into human medicine [9].

An emerging alternative to the simplified stimuli utilized in traditional in vitro systems is the direct application of plasma or serum obtained from diseased individuals to cultured cells (e.g., vascular endothelial cells) [10, 11]. Previous studies have demonstrated unique results upon applying plasma, indicating that a net effect of specific stimuli and the presence of blood components (albumin, complement factors, protease inhibitors, etc.) unaccounted for in more simplified stimuli, such as "cytomix," likely induces different results in vitro [5, 6]. Importantly, while still a relatively uncommon method, human plasma and serum have been used as a stimulus to study disease-specific conditions such as sepsis, cardiovascular and pulmonary diseases, SARS-CoV-2, diabetes, and cancer, as well as potential pharmacological treatments in cell culture [2, 3, 6, 9–12].

The purpose of this review is to discuss the application of either plasma or serum as a stimulus in cell culture models to assess physiological responses, disease mechanisms, and pharmacological treatments (Figure 1). Methodological processes, alternative applications of plasma versus serum for in vitro models, and future directions will also be discussed.

2 | Methodological Considerations

Due to the differences between plasma and serum, we examine their respective suitability for cell culture experiments, highlighting key considerations for ensuring consistent sample matching. Additionally, we address the methodological complexities associated with specific experimental objectives and discuss potential limitations.

2.1 | Human Plasma Versus Human Serum

As highlighted in the literature, both plasma (Table 1) and serum (Table 2) have proven to be adequate stimuli for the investigation of a variety of diseases and pharmacological mechanisms. However, the two blood components vary in their composition, potentially influencing experimental results. As compared to serum, plasma contains a greater abundance of fibrinogen and various coagulation factors [56]. Inflammatory mediators/cytokines, which may induce more physiologically relevant in vitro cellular responses, are variable between samples depending on the analyte being measured [57]. However, plasma application to cell culture has been reported to impair cell viability and to induce clotting of cell culture medium when applied at higher concentrations [58].

The most used plasma anticoagulants are ethylenediaminetetraacetic acid (EDTA), citrate, and heparin [59]. EDTA and citrate prevent coagulation through the chelation of divalent cations, such as calcium and magnesium [59]. A critical limitation of these two anticoagulants in cell culture experiments; however, is that their clotting prevention can be overcome by the divalent cations present in cell culture medium [59]. Heparin functions by inhibiting the ability of thrombin to convert fibrinogen into fibrin [60], resulting in continued anticoagulation that is independent of the media composition. Heparin has a highly negative charge; however, this can decrease cellular viability, disrupt cell adhesion, alter cellular responsiveness, and interfere with signaling [61, 62]. Therefore, when using heparin in cell culture, it should be administered at a minimum concentration of 0.25 IU/mL, the concentration able to prevent coagulation in 1 mL of blood, to minimize any off-target effects and reduce potential variability [63]. Ultimately, the choice of anticoagulant will depend on the experimental paradigm, with consideration of potential anticoagulant interference.

Both plasma and serum contain complement factors, which may be cytotoxic in vitro depending on the cell type investigated (e.g., Neuronal cells) [64]. For this reason, fetal bovine serum (FBS), an abundant source of growth factors that is frequently added to cell culture, may undergo heat inactivation to prevent the action of complement proteins [65]. However, heat inactivation of human plasma/serum for cell culture experiments (particularly related to inflammatory activation) would deactivate cytokines and other proteins involved in disease pathology [66]. Similarly, the application of antibiotics in cell culture media may pose similar challenges, particularly through binding to proteins such as albumin [67, 68].

2.2 | Plasma/Serum Dilutions for In Vitro Experiments

In the context of plasma/serum concentration relative to culture media, it is also important to consider the intentions of the study as well as any potential methodological alterations. Plasma/serum

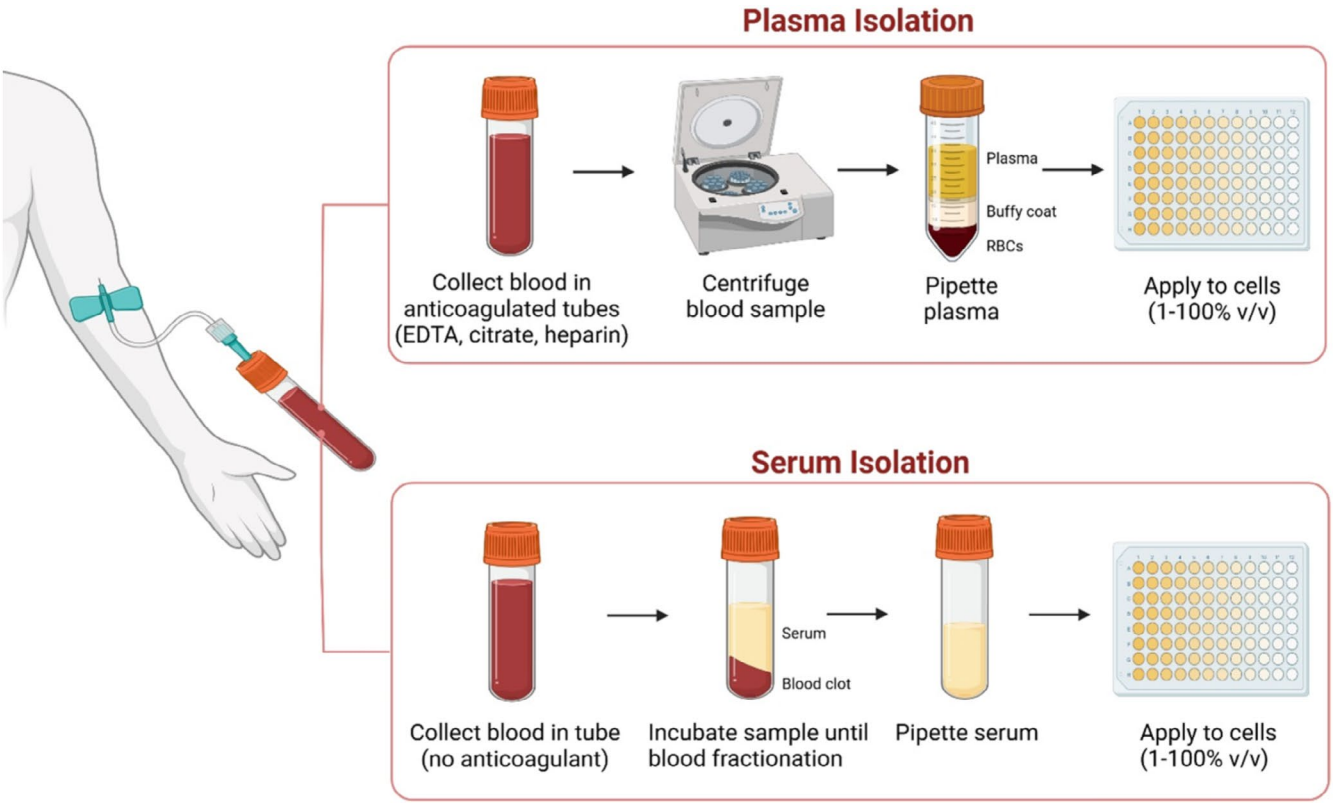

FIGURE 1 | Schematic representation of the process for obtaining plasma or serum from human whole blood using specific vacutainer tubes and centrifugation. Blood collection tubes are loaded with an anticoagulant of choice [ethylenediaminetetraacetic acid (EDTA), citrate, or heparin] for plasma collection, or without anticoagulants for serum collection. Centrifugation fractionates anticoagulated whole blood into red blood cells (RBCs), a buffy coat layer containing leukocytes/thrombocytes, and plasma. In the absence of anticoagulants, blood is fractionated into a clot and serum. Plasma or serum samples can be applied directly to cell cultures at the desired concentration relative to the culture media. Illustration created with BioRender.com.

concentration appears to vary greatly between different studies, from 1% to 2% (v/v) [12, 43] up to 50%–100% [13, 15]. The use of high concentrations (≥ 20%), which are particularly relevant to cells directly exposed to blood (e.g., endothelial cells), leads to a higher level of cell stimulation over a shorter timeframe (e.g., 1–12 h) [13, 24, 33]. For example, 20% (v/v) plasma has been shown to induce notable endothelial barrier hyper-permeability as assessed in a dose-dependent manner over a period of 8 h [6]. Similarly, 20% septic serum was shown to induce endothelial cell detachment in a shear stress model at a 2-h time point [24].

In contrast, the use of lower plasma/serum concentrations (≤ 5%) allows for the observation of effects over extended time periods as well as mimicking the concentration of plasma in tissues, which are not directly exposed to systemic circulation [23, 30]. For example, stimulation of myotubes with 5% plasma in media significantly increased myotube diameter at 24 and 48 h post-stimulation, with no significant effect present at 2 h post-stimulation [23]. Additionally, the use of undiluted plasma or higher concentrations of plasma has a greater tendency to coagulate, which then requires the addition of higher amounts of anticoagulants [10] or induces complement-mediated cytotoxic effects requiring prior heat inactivation [65]. The addition of increased anticoagulants or heat-inactivated plasma/serum may decrease the reliability of the results, as described above. Overall, it appears that the concentration of plasma or serum used for cell culture experiments may depend on the disease/pathology investigated, cell type, and even specific objectives of the study, with a concentration of 20% appearing to be the optimal choice for maintaining the balance between expected cellular responses and accounting for resource management [6, 10].

2.3 | Investigated Mechanisms

Many different cellular responses and mechanisms related to alterations in cell morphology, as well as functional correlates such as cell migration, proliferation, and adhesive properties, have been investigated using human plasma or serum as a cell culture stimulus [9, 14, 50]. Key cell-activating and inflammatory response-modulating factors (e.g., production of reactive oxygen species [ROS] and nitric oxide [NO]) in response to cell stimulation with plasma or serum are presented in Table 3. In addition, the use of plasma as a stimulus and the subsequent characterization of that plasma through techniques such as immunoassays will aid identification of key pathological components within the plasma that contribute to alterations in cellular characteristics [10].

2.4 | Effects of Biological and Lifestyle Factors

Pooled samples have been used to obtain consistent results, as opposed to comparing samples from individual subjects matched by age and gender [30, 47, 51]. This approach assumes

TABLE 1 | Publications utilizing plasma for in vitro cellular studies.

Publication	Model	Cell type	Concentration (% v/v)	Duration	Coagulant
[13]	Sepsis	MCVEC	100%, 50%, 20%, 10%	1 h	Heparin
[14]	Sepsis	HUVEC*	10%	4 h	Heparin
[6]	Sepsis	PMVEC*, AEC*	20%	8 h	N/A
[15]	Sepsis	HUVEC*	50%	0.5 h	EDTA
[16]	Sepsis	HSPC*	20%	24 h	EDTA
[17]	Sepsis	C2C12	5%	24 h	N/A
[18]	COVID-19	HPMVEC*	N/A	1 h	Citrate
[19]	COVID-19	STC*, HTO*	20%	24 h	N/A
[10]	DKA	HCMEC*	20%	6 h	Heparin
[2]	COPD	MRC-5*, Nuli-1*	25%	24 h	N/A
[20]	COPD	Hep-2*, HAE*	2.50%	0.5 h	Heparin
[21]	Liver disease	Huh7.5*, PHH*	5%, 10%, 20%	3 h	N/A
[22]	Tissue regeneration	hOB*	N/A	48 h	EDTA/citrate
[23]	Age effects	C2C12	5%	48 h	Heparin

Note: Asterisks (*) indicate human cell lines.
Abbreviations: AEC, primary human alveolar epithelial cells; C2C12, myoblasts; HAE, human airway epithelial cells; hCMEC, human cerebral microvascular endothelial cells; Hep-2, human epithelial cells; hOB, human osteoblasts; HPMVEC, human pulmonary microvascular endothelial cells; HSPC, human hematopoietic stem and progenitor cells; HTO, 3D human testicular organoids; Huh7.5, human hepatocellular carcinoma cell line; HUVEC, human umbilical vein endothelial cells; MCVEC, mouse cerebrovascular endothelial cells; MRC-5, human lung fibroblast cell line; N/A, not available; Nuli-1, human airway epithelial cell line; PHH, primary human hepatocytes; PMVEC, pulmonary microvascular endothelial cells; STC, mixed seminiferous tubule cells.

that the composition of the plasma and serum is consistent among age and sex. Indeed, changes in myoblast proliferation and differentiation, when stimulated by sera from either young or old donors, showed that donor age did not cause any noticeable differences [52]. In contrast, both myotube diameter and muscle protein synthesis were lower when stimulated with sera from older people than with sera from younger people [53]. Additionally, alterations in the activation of human pulmonary arterial adventitial fibroblasts (hPAAF) and the apoptosis of human hippocampal progenitor cells (HPCs) have been observed when comparing sera from different ages and sexes [54, 55]. Metabolomic profiling of serum indicates differences in metabolite expression levels according to age and sex [69–71], thus age and sex matching of donors may reduce possible confounding variables. Variations in immune responses related to age and sex also contribute to differences in inflammatory cytokine and chemokine production [72–74]. Hormonal differences play a significant role, with estrogen enhancing humoral immunity and increasing inflammation susceptibility [72, 75]. Aging is associated with heightened inflammatory responses, including increased production of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [76–78]. Furthermore, variations in serum albumin concentration, a key component in cell growth and proliferation [79], show a decline with age and a more pronounced decrease in older individuals [80]. These variations can pose challenges for studies investigating cellular mechanisms or employing serum-free media for treatments.

When using plasma and serum as stimuli in cell culture experiments, it is important to consider the variability between individuals due to biological and lifestyle factors, as well as the use of healthy volunteers as controls. Healthy control subjects are utilized to assess the effects of plasma/serum in vitro while minimizing confounding variables related to disease factors [12]. However, several elements can influence plasma and serum composition. For instance, alterations in the plasma proteome have been linked to higher body mass index (BMI), including increased levels of leptin and fatty acid-binding protein-4 (FABP4), and decreased levels of sex hormone-binding globulin (SHBG) [81]. Additionally, oxidative stress, indicated by elevated plasma protein carbonyls, has been associated with alcohol consumption and cigarette smoking [82]. Medications such as antihypertensives and lipid-lowering drugs can also impact the abundance of approximately 35.7% of plasma proteins [83].

Dietary factors and exercise also affect serum composition. For example, serum from fed volunteers has been shown to significantly increase muscle cell protein synthesis compared to serum from fasted individuals [43–46]. Furthermore, serum from aerobically exercised subjects can induce changes in muscle cell cultures, such as increased myogenesis, compared to serum from untrained individuals [47]. Additionally, serum from exercised patients has demonstrated inhibitory effects on cancer cell proliferation and viability, with increases in p53 content and reductions in nuclear factor kappa beta (NFkB) activation [48], as well as decreased phosphorylation and activation of key kinases, including mammalian target of rapamycin (mTOR), protein kinase B (Akt), extracellular signal-regulated kinases (Erk1/2), and ribosomal protein S6 kinase B1 (p70 S6K) [50]. Increased effects of cancer-inhibitory myokines, such as osteonectin and oncostatin M, were also observed with serum from exercised

TABLE 2 | Publications utilizing serum for in vitro cellular studies.

Publication	Model	Cell type	Concentration (% v/v)	Duration
[24]	Sepsis	HUVEC*	20%	2 h
[25]	Sepsis	HUVEC*, HMVEC*	5%	0.5 h
[26]	Sepsis	CEM*, Raji*, K562*	10%	48 h
[27]	Sepsis	Monocytes*	N/A	18 h
[28]	Sepsis	Myocardial cells	10%	N/A
[11]	COVID-19	HUVEC*	10%	24 h
[29]	COVID-19	PBMC*	50%	2 h
[30]	COPD	Myoblasts*	2%	120 h
[9]	COPD	HUVEC*	50%	20 h
[31]	COPD	PBMC*, MH-S	1%, 5%, 10%	6 h
[32]	Smoking	HCAEC*	50%	12 h
[33]	Smoking	HUVEC*	50%	12 h
[34]	Liver disease	C2C12	10%	4 h, 24 h
[35]	Liver disease	PHH*	2.50%	2–16 h
[36]	Liver disease	CD4+ T cells*	10%	24–48 h
[37]	Tissue regeneration	hCSC*	10%	48 h
[38]	Tissue regeneration	TIG-1*, skin fibroblasts*	10%	48 h
[39]	Tissue regeneration	TIG-3S*	10%	21 h
[40]	Mesenchymal stem cells	hMSC*	10%	120 h
[3]	Rituximab	Daudi cells*	10%	24–28 h
[41]	Rituximab	Primary NHLC*	2%, 10%, 30%, 50%	2 h
[12]	Cancer	BLEC*	2%	24 h
[42]	Cancer	LoVo*	10%	48 h
[43]	Fed vs. fasted	L6-GLUT4myc	1%	1 h
[44]	Fed vs. fasted	C2C12	10%, 20%, 50%, 100%	1–4 h
[45]	Fed vs. fasted	C2C12	20%	4 h
[46]	Fed vs. fasted	C2C12	20%	4 h
[47]	Exercise	LHCN-M2*	0.50%	96 h
[48]	Exercise	LNCaP*, PC-3*	10%	48 h
[49]	Exercise	LNCaP*, PC-3*	5%	96 h
[50]	Exercise	A549*, MRC5*	10%	72 h
[51]	Burn injury	Satellite cells*	5%	48 h, 96 h
[52]	Age effects	Satellite cells*	2%, 15%	46 h
[53]	Age effects	C2C12	10%	4 h, 24 h
[54]	Age effects	HPCs*	1%	48 h
[55]	Age and sex effects	hPAAFs*	1%	168 h

Note: Asterisks (*) indicate human cell lines.
Abbreviations: A549, human lung adenocarcinoma cells; BLEC, brain-like endothelial cells; C2C12, myoblasts; CEM, human T lymphoid cell line; hCAEC, human coronary artery endothelial cells; hCSC, human cardiac stem cells; hMSC, human mesenchymal stem cells; HMVEC, human microvascular endothelial cells; HPAAF, human pulmonary arterial adventitial fibroblasts; HPCs, human hippocampal progenitor cells; HUVEC, human umbilical vein endothelial cells; K562, human cell line derivate of chronic myelogenous leukemia; L6-GLUT4myc, rat myoblast cell line; LHCN-M2, immortalized human skeletal myoblasts; LNCaP, androgen-sensitive human prostate adenocarcinoma cells; LoVo, human colon cancer cell line; MH-S, murine alveolar macrophage cell line; MRC5, normal lung fibroblasts; N/A, not available; NHLC, non-hodgkin lymphoma cells; PBMC, peripheral blood mononuclear cells; PC-3, human prostate cancer cell line; PHH, primary human hepatocytes; Raji, human B lymphoid cell line; TIG-1, human fetal lung fibroblasts; TIG-3S, human fetal skin fibroblasts.

TABLE 3 | Cellular mechanisms investigated in vitro with human plasma or serum.

Publication	Model	Signal transduction	Function
[13]	Sepsis	↑ROS, ↑NO / ↓occludin, tight junction protein	↑permeability
[14]	Sepsis	↑ cytokine/chemokine release, ICAM-1	↑ monocyte adhesion
[6]	Sepsis	↑ Il-6, IL-8, MIP1B, IP10, MCP1, GCSF	PMVEC hyper-permeability
[15]	Sepsis	↑ ROS	N/A
[24]	Sepsis	N/A	↑ stress fiber induction, ↓adherence
[25]	Sepsis	Ang2/Tie2 signaling	Thinning of the HUVEC glycocalyx layer
[16]	Sepsis	↑IL-7	↑myelopoiesis, MS1 gene expression
[17]	Sepsis	↑ IL-6, MuRF-1, MAFbx, NFKB, ubiquitinated myosin	↓ in myosin content of myotubes
[26]	Sepsis	↑ glucocorticoid receptor alpha/beta expression	N/A
[27]	Sepsis	↑TREM-1 receptor/ligand	N/A
[28]	Sepsis	Myocardial cell depression via TNF-α and Il-1B	N/A
[11]	COVID-19	↑GPX4, SLC7A11, FTH1, SAT1, ROS	↑Ferroptosis
[18]	COVID-19	N/A	↓viability upon exposure to COVID-19 plasma
[19]	COVID-19	N/A	↓ viability, death of undifferentiated spermatogonia
[29]	COVID-19	↑S100A elevation, ↓antigen presentation	Myeloid dysfunction
[10]	DKA	↑ROS	↑ neutrophil adhesion
[30]	COPD	N/A	↓ myotube diameter, pooled serum benefits
[9]	COPD	↑IL-6, MCP-1	↓ endothelial migration
[32]	Smoking	↑ROS, ↓NO	N/A
[33]	Smoking	↓NO	N/A
[2]	COPD	↑ CRP and Il-8, ↓ MMP-9	N/A
[20]	COPD	↑antibodies	↑ cytotoxicity
[31]	COPD	↑ CCL-5 and TNF-α, ↓ Il-10	N/A
[34]	Liver disease	N/A	↓ coupling efficiency, mitochondrial respiration, mitophagy, and diameter
[21]	Liver disease	↑ SREBP-1c, PPARγ, NF-kB, NOX2, ROS	↓ cell viability and mitochondrial membrane potential
[35]	Liver disease	N/A	HCV infectivity was greatest during the first 3 days.
[36]	Liver disease	↑ CTLA4 and soluble B7, ↓ response to antigen and CD3 stimulation	N/A
[22]	Tissue regeneration	↑ VEGF, HGF, procollagen I, osteocalcin, and alkaline phosphatase	↑migration, proliferation, chemotaxis of osteoblasts
[37]	Tissue regeneration	↑ p38MAPKand Hsp27 phosphorylation	↑migratory capacity of hCSC

(Continues)

TABLE 3 | (Continued)

Publication	Model	Signal transduction	Function
[38]	Tissue regeneration	N/A	Inhibition of fibroblast migration
[39]	Tissue regeneration	N/A	Inhibition of fibroblast migration
[40]	Mesenchymal stem cells	↑ alkaline phosphatase activity and 25-Hydroxyvitamin D	↑MSC proliferation and differentiation
[3]	Rituximab	N/A	↓ Daudi lymphoma cell proliferation
[41]	Rituximab	N/A	Cytotoxicity
[12]	Cancer	↑ CXCL13 and TJ protein occludin expression	↑ permeability
[42]	Cancer	↑ y-H2AX expression, Il-6	Reduced proliferation
[43]	Fed vs. fasted	↑GLUT 4 translocation	N/A
[44]	Fed vs. fasted	↑ mTOR, p70S6K,4E-BP1 activation	↑ muscle protein synthesis (fed>fasted serum)
[45]	Fed vs. fasted	N/A	↑ muscle protein synthesis (fed>fasted serum)
[46]	Fed vs. fasted	↑ P70S6K, mTOR and 4E-BP1 physphorylation	N/A
[50]	Exercise effects	↓ Akt, mTOR, p70S6K and ERK1/2	↓ cell proliferation and survival
[47]	Exercise effects	↓ apoptotic factors P62 and Bcl-2	↑myotube formation and differentiation
[48]	Exercise effects	↑ p53 content, ↓ NFkB activity	N/A
[49]	Exercise effects	↑ oncostatin M and osteonectin, testosterone	↓ cell metabolic activity
[51]	Burn injury	↑ STAT3 phosphorylation	↓ myogenesis, myogenic fusion signaling and myogenin
[23]	Age effects	↑ FLRG and activin A for older plasma	↑ scratch closure, ↑ myotube diameter
[52]	Age effects	N/A	No noticeable difference between young and old patient sera
[53]	Age effects	N/A	↓ myotube diameter, muscle protein synthesis with old serum
[54]	Age effects	↑ apoptotic factors	Cell apoptosis
[55]	Age and sex effects	↑ cytokine, chemokine release	Variation in cell activation

Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; Akt, protein kinase B; Bcl-2, B-cell lymphoma 2; CCL-5, C-C motif chemokine ligand 5; CD-3, Cluster of differentiation 3; CRP, C-reactive protein; CTLA4, Cytotoxic T-lymphocyte associated protein 4; CXCL13, Chemokine ligand 13; Erk1/2, extracellular signal-regulated kinase 1/2; FLRG, follistatin-related gene; GLUT4, Glucose transporter type 4; HGF, hepatocyte growth factor; Hsp27, heat shock protein 27; ICAM-1, Intercellular adhesion molecule 1; Il-10, Interleukin 10; IL-1B, Interleukin 1 beta; IL-6, Interleukin6; IL-7, Interleukin 7; Il-8; Interleukin 8; IP-10, interferon gamma-induced protein 10; MAFbx, Atrogin-1; MAPK, Mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein-1; MIP1B, macrophage inflammatory protein 1 beta; MMP-9, Matrix metalloproteinase 9; MS1, CD14 monocyte state; mTOR, mammalian target of rapamycin; MuRF-1, Muscle RING-finger protein 1; N/A, not available; NFKB, Nuclear factor kappa B; NO, nitric oxide; NOX2, NADPH oxidase 2; p38MAPK, p38 mitogen-activated protein kinase; p53, Tumor protein p53; p62, Sequestosome-1; P70 S6K, ribosomal protein S6 kinase B1; PPARy, Peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; S100A, alarmin family genes; SREBP-1c, Sterol regulatory element binding protein-1c; STAT3, Signal transducer and activator of transcription 3; TNF, Tumor necrosis factor; TREM-1, Triggering receptor expressed on myeloid cells 1; VEGF, vascular endothelial growth factor; y-H2AX, histone family member.

individuals [49]. These findings underscore the necessity of accounting for biological and lifestyle factors when interpreting experimental results.

2.5 | Challenges and Limitations

While human plasma and serum are valuable tools in cell culture research, several challenges must be addressed. As noted earlier, plasma and serum samples exhibit inherent variability between individuals, necessitating careful matching across cohorts. In addition to the high costs associated with obtaining and storing these samples, ethical concerns surrounding the use of human-derived materials further complicate their feasibility. Consequently, the application of this technique remains limited in the current literature. From a technical standpoint, the required dilutions of plasma and serum in cell culture reduce the concentrations of key mediators involved in disease pathology,

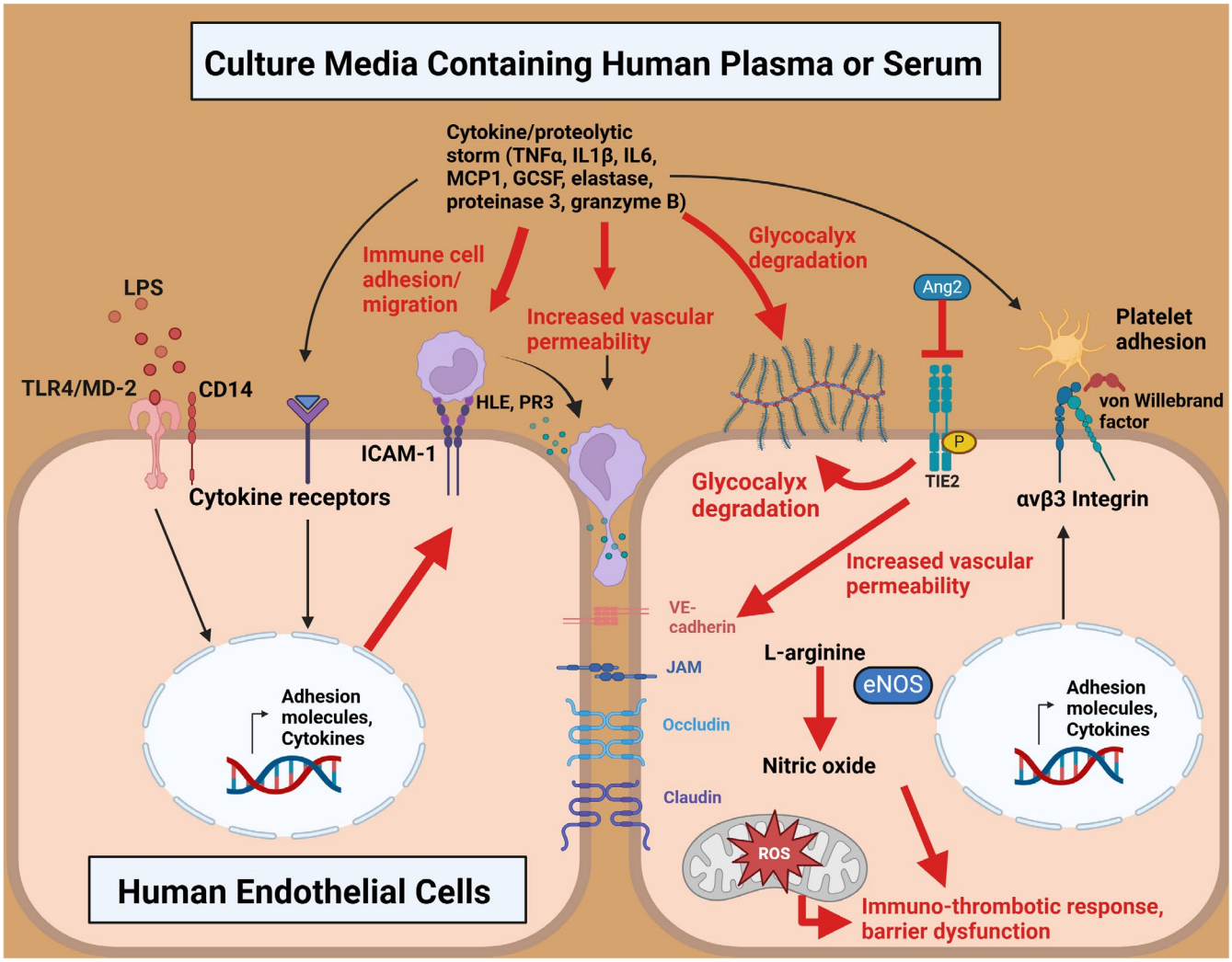

FIGURE 2 | Cell signaling and functional changes induced by septic plasma/serum stimulation in vitro. The schematic illustrates how septic plasma/serum promotes (induces) inflammatory activation in endothelial and/or immune cells. This activation is driven by the combined effects of LPS, cytokines, and proteolytic enzymes present in the plasma/serum. The net effect includes oxidative and nitrosative stress, upregulates pro-adhesive phenotype, and dysfunction of the endothelial cell barrier, resulting in increased permeability. Red arrows highlight mechanisms investigated in the literature using septic human plasma/serum [6, 13–15, 25], while black arrows denote other septic signaling pathways. Abbreviations: TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein-1; G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; TLR-4, toll-like receptor 4; MD-2, myeloid differentiation factor 2; CD14, cluster of differentiation 14; NFκB, nuclear factor kappa B; ICAM-1, intercellular adhesion molecule 1; Ang2, angiopoietin-2; TIE2, tunica intima endothelial kinase 2; JAM, junctional adhesion molecule; eNOS, endothelial nitric oxide synthase; HLE, human leukocyte elastase; PR3, proteinase 3. Illustration created with BioRender.com.

potentially attenuating physiological responses and limiting the relevance of experimental outcomes [10].

3 | Disease-Related Studies

3.1 | Sepsis

The use of patient plasma and serum as a stimulus for cell culture experiments has largely been directed at the effects of septic conditions (e.g., proven or suspected infection with a non-specific systemic inflammatory response). Endothelial cell activation is a primary focus of sepsis experiments (Figure 2) due to the overwhelming recruitment of immune cells and increased vascular permeability [14]. Stimulating human pulmonary microvascular endothelial cells (HPMVEC) in culture media for 8 h with 20% plasma obtained from septic patients increased microvascular permeability as compared to stimulation with plasma obtained from healthy control subjects [6]. When cells were stimulated with plasma from septic patients, LPS, or cytomix (an equal mix of interferon gamma (IFNγ), IL-1β, and TNF-α), the effects on permeability were similar [6]. A different study used 10% septic plasma to stimulate human umbilical vein endothelial cells (HUVEC) for 4 h. Plasma was either untreated or incubated with polystyrene-divinylbenzene-based adsorbents for cytokine adsorption [14]. Monocyte adhesion to HUVEC stimulated with untreated plasma was significantly increased, whereas adhesion returned to baseline levels for HUVECs stimulated with cytokine-depleted plasma [14]. Furthermore, a correlation was drawn between elevated monocyte chemotactic protein-1 (MCP-1) and granulocyte colony-stimulating

factor (G-CSF), as well as increased endothelial activation leading to increased monocyte adhesion following plasma stimulation [14]. Acute (30 min) stimulation of HUVEC with human septic plasma (50% v/v) resulted in higher production of ROS as compared to HUVEC treated with plasma from healthy volunteers [15]. Interestingly, the production of ROS was maximal within 10 min of exposure of HUVEC to plasma and returned to baseline immediately following plasma removal from the cell culture [15]. Furthermore, a direct link was found between the sepsis severity of the illness and a rise in ROS production, indicating that the severity/progression of the illness may be another factor to be considered when investigating the effects of stimulation with plasma and serum [15].

Beyond endothelial cells, it was shown that plasma from sepsis patients induced myelopoiesis in bone marrow hematopoietic stem and progenitor cells (HSPCs) and expanded an IL-6-associated CD14+ monocyte state [16]. IL-6 levels in plasma obtained from sepsis patients early in their disease state correlated with a decrease in the myosin content of myotubes after incubation with 5% plasma for 24 h, indicating skeletal muscle atrophy in vitro [17]. The addition of an IL-6 inhibitor to the culture media abrogated this latter observation, as did patient samples collected late in the disease process [17].

An investigation of septic conditions using human serum as a stimulus supports many of the plasma findings discussed above. Specifically, alterations in HUVEC morphology, such as stress fiber induction and decreased adherence upon exposure to shear stress, were observed following stimulation with 20% serum from sepsis patients in comparison to 20% autologous serum (28.7% septic vs. 96.8% healthy HUVEC adhered) [24]. The HUVEC glycocalyx, a specialized extracellular matrix that covers the apical side of vascular endothelial cells [84], became thinner when 5% serum from septic patients was added to the culture media [25]. The latter change was found to be dependent on Tie2 (an endothelium-stabilizing receptor tyrosine kinase) deactivation, suggesting that the activation of Tie2 may be a means of preventing this outcome, as well as demonstrating the applicability of human serum for examining mechanisms of disease through composition analysis [25].

Human serum has been used as a stimulus for investigating ligand-receptor interactions in the context of sepsis. Exposing human immune cell lines CEM (T lymphocytes), Raji (B lymphocytes), and K562 (a derivate of chronic myelogenous leukemia) to 10% serum from sepsis patients for 48 h increased both glucocorticoid receptor alpha and beta expression in CEM and Raji cells but, paradoxically, downregulated the expression of both receptors in K562 cells [26]. Also, septic patient serum was found to contain high concentrations of the triggering receptor expressed on myeloid cells 1 (TREM1) ligand, associated with TREM1 receptor expression in monocytes in comparison to healthy controls [27]. Additionally, serum from sepsis patients showed increased TNF-α and IL-1β expression, and when 10% serum was applied to cultured myocardial cells, their contraction was depressed [28]. Cytokine depletion from the serum reversed the myocardial depression [28]. Collectively, these studies describe phenotypic and intracellular alterations in cell culture in response to human plasma or serum and identify pathogenic components.

3.2 | SARS-CoV-2

Recent studies successfully employed both plasma and serum obtained from COVID-19 patients to address diagnostic, prognostic, and pathophysiological mechanisms. Exposing HUVEC to 10% serum from COVID-19 patients (survivors vs. non-survivors) for 24 h induced greater mitochondrial and cellular ROS production, lipid peroxidation, as well as TNF-α-mediated ferroptosis [11]. The impact of COVID-19 illness severity was assessed on inducing endothelial cell damage in vitro, as evidence suggests that damaged endothelial cells contribute to microvascular thrombosis commonly seen in severe cases of COVID-19 [18]. HPMVEC had significantly reduced viability upon exposure to COVID-19 plasma for 1 h, whereas HPMVEC viability was not impaired by plasma from COVID-19 patients that had recovered [18]. Mixed seminiferous tubule cells and 3D human testicular organoids lost their viability after being stimulated with 20% COVID-19 plasma for 24 h, as well as a corresponding increase in the death of undifferentiated spermatogonia [19]. The severity of the multisystem inflammatory syndrome in children (MIS-C), which occurs post-SARS-CoV-2 infection, has also been investigated by stimulating human peripheral blood mononuclear cells (PBMCs) with 50% MIS-C serum for 2 h [29]. An elevation of S100 calcium-binding protein A (S100A) family alarmins was observed, suggesting myeloid dysfunction, as well as a decrease in antigen presentation [29].

3.3 | Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is associated with hyperglycemia, ketoacidosis, and systemic inflammation [10, 85–87]. To date, only a single study has investigated DKA plasma-induced inflammatory effects/mechanisms in human-derived cells in culture [10]. In this study, leukocyte-cerebrovascular endothelial cell (hCMEC) activation and subsequent adhesive interactions were assessed using plasma from age- and sex-matched and insulin-controlled DKA patients [10]. In addition, both cytokine and chemokine concentrations were measured to create a DKA-specific cytomix [10]. The hCMECs were stimulated with either 20% DKA plasma, containing heparin (1 U/mL) as an anticoagulant, or the DKA cytomix, leading to polymorphonuclear neutrophil adhesion under the condition of flow [10]. Additionally, in support of the previous findings addressing the effects of sepsis-plasma on endothelial cell activation/ROS production [13, 15], DKA plasma was able to induce oxidative stress in hCMEC; however, this was not seen with the addition of DKA cytomix [10]. The inability of the cytomix solution to induce oxidative stress indicates that DKA plasma (even used at 20% v/v concentration) contains higher oxidative-stress inducing potential, thus supporting the use of plasma (and potentially serum, as well) as a stimulus for the further improvement of disease-specific model(s) in vitro [10].

3.4 | Chronic Obstructive Pulmonary Disease

While cultured endothelial cells have been exposed to cigarette smoke to study chronic obstructive pulmonary disease (COPD),

this approach may not be physiologically relevant since endothelial cells in vivo do not get directly exposed to cigarette smoke [9]. To overcome this limitation, HUVEC were stimulated for 20 h with 50% serum from smokers and non-smokers [9]. Serum from smokers inhibited endothelial cell migration in a damage repair model and increased gene expression associated with cardiovascular disease [9]. Moreover, treatment of human coronary artery endothelial cells (HCAECs) and HUVECs for 12 h with the same concentration of serum led to significantly higher endothelial nitric oxide synthase (eNOS) expression in both cell types; however, eNOS activity and nitric oxide production were significantly reduced [32, 33]. In addition, lung fibroblast cells and cells of the airway lining showed a significant increase in C-reactive protein (CRP) and interleukin 8 (IL-8) following stimulation for 24 h with 25% plasma from COPD patients as compared to healthy controls [2]. Anti-pulmonary airway epithelial cell antibodies are present in COPD plasma compared to healthy plasma, and the cytotoxic effects of COPD plasma on PBMC-epithelial cell co-cultures were observed compared to incubation with plasma from non-COPD subjects [20].

COPD inflammation was modeled by using diseased serum at various concentrations and PBMCs from patients with different disease severities [31]. Increasing disease severity was correlated with increased levels of CCL5 and TNF-α, as well as decreased IL-10 in COPD serum [31]. This pattern of expression was also seen in murine alveolar macrophage (MH-S) cells that were cultured and stimulated with 1% COPD serum instead of 1% healthy serum, and increasing concentrations of COPD serum (5% and 10%) induced a greater magnitude of inflammatory gene expression [31]. The effects of COPD were further expanded to myoblasts stimulated with COPD serum at a 2% concentration in culture media over 5 days, demonstrating the reliability of this method for investigating disease mechanisms over extended periods of time [30]. Significantly decreased surface area covered by myotubes was noted, as well as a reduction in diameter [30]. Pooled serum was used as a means of reducing variations based on individual serum samples, demonstrating the atrophying effects on healthy myotubes [30].

3.5 | Liver Disease and Hepatocyte Dysfunction

To investigate sarcopenia in liver disease in vitro, C2C12 myotubes were stimulated with serum from four non-alcoholic fatty liver disease (NAFLD) patients, four end-stage liver disease (ESLD) patients, and four age-matched healthy controls [34]. Results were measured at 4 and 24 h post-stimulation with 10% serum in growth media [34]. When myotubes were stimulated with NAFLD and ESLD serum, coupling efficiency, mitochondrial respiration, and mitophagy decreased compared to healthy control serum [34]. Also, when myotubes were stimulated with ESLD serum instead of NAFLD or control serum, their diameter was reduced [34]. The effects of NAFLD plasma have also been explored in cultured Huh7.5 cells and primary human hepatocytes at concentrations of 5%, 10%, and 20% [21]. NAFLD plasma stimulation led to lower cell viability and higher ROS production in both types of cells [21]. The effects were stronger at higher plasma concentrations [21].

Hepatitis C (HCV) has been investigated in primary human hepatocytes (PHHs) cultured in 2.5% HCV-positive serum for 2–16 h [35]. Viral infection was determined after HCV-serum stimulation, and the number of HCV RNA copies was determined using reverse transcription and real-time PCR analysis [35]. HCV infectivity was greatest during the first 3 days after the seeding of PHHs, and HCV RNA concentrations were still present at 16 days post-seeding for highly infectious sera [35]. Quantification of serum revealed that the highly infectious cohort contained lower levels of 24 analytes related to hepatocyte biology and infection efficiency, suggesting that the combination of these analytes was directly related to serum infectivity [35].

To investigate cellular reactions to acute liver failure (ALF), CD4+ T lymphocytes were grown in a medium that had 10% ALF serum or healthy control serum for up to 48 h [36]. The use of flow cytometry revealed that T lymphocytes exposed to ALF sera expressed higher levels of cytotoxic T-lymphocyte associated protein 4 (CTLA4), a protein that downregulates the immune response of T lymphocytes [36]. This increase in CTLA4 was due to significantly increased levels of sB7 in serum from ALF patients [36].

4 | Therapeutic Studies

4.1 | Tissue Regeneration

Tissue regeneration is largely driven by the migratory potential of stem cells, which play a crucial role in wound healing [37]. Analysis of primary human cardiac stem cell (hCSC) migration after stimulation for 48 h with 10% human serum demonstrated increased speed and distance of hCSC migration versus serum-free medium [37]. In addition, human plasma, applied to osteoblasts in cell culture, was used to investigate their adhesive and migratory functions. It was determined that plasma rich in growth factors (PRGF) could promote the regeneration of bone tissue in vitro through increased osteoblast proliferation, migration, and chemotaxis [22]. However, in contrast to these findings, serum at 10% v/v inhibited the migration of fibroblasts derived from fetal skin and lung [38, 39], suggesting that the effects of serum on cell migration may vary depending on cell type. Finally, human serum has also been used to induce osteogenic differentiation of human mesenchymal stem cells, an important component of bone regeneration [40]. In this study, cells were cultured in media containing 10% human serum for 5 days, resulting in increased osteogenic differentiation, retained marker expression, and higher alkaline phosphatase activity [40]. Elevated levels of osteogenic components, such as 25-hydroxyvitamin D and alkaline phosphatase in human serum, were suggested as potential mechanisms for increased osteogenic differentiation [40].

4.2 | Cancer and Pharmaceutical Therapies

While plasma and serum are useful components for investigating disease mechanisms in vitro, they have also been used to further our knowledge of currently used therapeutics. For example, the monoclonal antibody rituximab has anti-tumor activity in non-Hodgkin's lymphoma patients [3], and when Daudi lymphoma cells were stimulated for 24–48 h with 10% autologous serum containing 100 μg/mL rituximab, cell growth was directly inhibited [3]. Similarly, lymphoma cell viability was assessed with graded doses of rituximab incubated for 2 h in the

presence of increasing amounts of human serum (2%, 10%, 30%, and 50%) [41]. The percentage of viable cells was lower at higher concentrations of serum, suggesting the presence of serum factors instigating cell death [41].

In another model, 2% sera from breast cancer patients were incubated for 24 h with human brain-like endothelial cells (BLECs) to investigate the effects of serum factors on the permeability of the human blood–brain barrier (BBB) [12]. Various chemokines were found to be elevated in breast cancer serum in comparison to healthy controls and were subsequently found to significantly increase endothelial monolayer permeability in BLECs [12]. This concept is also seen by stimulating a human colon cancer cell line (LoVo) with sera collected from the patients post-exercise and comparing it to a non-exercise control [42]. Cells were incubated for 48 h with 10% serum in media, and a reduction in LoVo cell proliferation was observed in cells stimulated with serum from patients who exercised [42]. Cytokine analysis of post-exercise serum showed an increase in IL-6 concentration [42].

5 | Clinical Interpretations

To date, the use of plasma and serum in cell culture has predominantly focused on investigating pathophysiological mechanisms underlying systemic disorders (Table 3). Specifically, these studies have explored the role of various mediators—such as cytokines, proteolytic enzymes, and redox-active molecules—in the activation of circulating immune cells, their interaction with the vascular endothelium, and the subsequent injury or dysfunction of the vasculature. Indeed, in vitro models have been employed to study conditions such as sepsis, COVID-19, chronic obstructive pulmonary disease (COPD), and diabetic ketoacidosis [6, 10, 11, 17, 31]. In these models, dysregulation of the redox system, including increased production of reactive oxygen species (ROS) and decreased nitric oxide (NO) levels, were commonly observed across the discussed pathologies. This redox imbalance often precedes endothelial dysfunction and vascular injury [13, 32]. In the context of DKA and sepsis, studies demonstrated enhanced neutrophil adhesion, along with increased cytokine and ROS production, suggesting a shift toward a pro-inflammatory endothelial phenotype associated with autoimmune conditions [10, 14]. Additionally, in sepsis and cancer models, increased endothelial permeability was observed, reflecting immune cell infiltration into tissues and the breakdown of the vascular barrier [6, 12, 13]. Collectively, the characterization of plasma and serum components in cell culture models provides valuable insights into disease-specific alterations in cellular signaling and function. This approach facilitates a better understanding of the molecular mechanisms driving disease progression, which is crucial for the development of diagnostic and prognostic platforms, as well as the identification of therapeutic targets and strategies.

6 | Future Directions and Considerations

Despite the overall benefits of using human plasma and serum to improve experimental settings and the translation of in vitro experimental work, there are several key factors to consider in planning future studies: (1) The concentration of plasma/serum to be investigated should be carefully chosen based on the cell type of interest [e.g., cells of the circulatory system such as leukocytes, platelets, and vascular endothelial cells should be treated with higher (> 20%v/v) plasma/serum concentrations, while parenchymal cells not directly interacting with blood (fibroblasts, myoblasts, etc.) should be treated with plasma/serum concentrations below 10% v/v] [10, 17]. These concentrations typically induce near-maximal cell-specific responses while conserving biological resources [10]. They also mitigate cytolytic effects due to complement factors and address issues related to anticoagulation [13, 62, 64]. (2) There is ongoing debate in the literature about the impact of physiological factors such as age and sex on cell culture outcomes [30, 47, 51–53]. Nevertheless, matching donors by age and sex in comparisons of diseased and healthy control subjects, alongside considerations of BMI, alcohol consumption, smoking habits, and medication use, can help account for variability. (3) Recent advancements in high-throughput technologies for measuring human blood proteins (e.g., Olink, Somalogic, and Alamar Biosciences) and metabolites (e.g., liquid chromatography with tandem mass spectrometry [LC–MS/MS] and nuclear magnetic resonance [NMR]) present robust opportunities to link the application of human plasma/serum to cell cultures with identified constituents, thus advancing future translational research [88]. (4) To further increase the translational relevance of in vitro cell culture experiments, the use of human cell lines in combination with human plasma/serum is strongly recommended [89]. (5) In line with the advancement of in vitro models (ex. organoids, organ-on-a-chip), the application of human plasma/serum demonstrates strong potential as a translational research approach to investigate disease mechanisms [90, 91].

Conflicts of Interest

The authors declare no conflicts of interest.

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