Comparison of different cell culture plates for the enrichment of non-adherent human mononuclear cells

ANNETT KLINDER[1], JANA MARKHOFF[1], ANIKA JONITZ-HEINCKE[1], PHILIPP STERNA[1], ACHIM SALAMON[2] and RAINER BADER[1]

[1]Department of Orthopaedics, Research Laboratory for Biomechanics and Implant Technology; [2]Department of Cell Biology, University Medicine Rostock, D-18057 Rostock, Germany

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Abstract. While tissue-resident monocytes and macrophages are considered to be vital players in the *in vivo* interaction between biomaterials and surrounding tissue, their isolation is limited. In order to establish *in vitro* models elucidating implant and tissue interactions, peripheral blood mononuclear cells (PBMCs) represent a viable source for bone marrow-derived monocytes and an alternative to tissue-resident cells. The aim of present study was to analyse different adhesion-preventing tissue culture plates for their potential to facilitate the culture of monocytes without differentiation into macrophages. Freshly isolated PBMCs were seeded into four commercially available tissue culture plates with different adhesive properties and were tested for surface CD14 and CD68 expression using flow cytometry following 7 days in culture. When PBMCs were cultivated in RPMI on Cellstar® Cell culture plates with Cell-Repellent Surface, a significant increase in CD14-positive cells was observed compared with cultivation in standard tissue culture-treated plates. This was accompanied by elevated cytokine production of interleukin-6 (IL6) and interleukin-8 (IL8); however, overall cell growth was not affected. When PBMCs were pre-cultured in cell-repellent plates, there was a higher yield of adherent cells after subsequent transfer into standard tissue culture-treated plates. Cultivation of PBMCs on cell-repellent culture plates favoured a monocytic phenotype and thus, represents an alternative to increase the fraction of monocytes yielded from PBMCs.

Correspondence to: Dr Annett Klinder, Department of Orthopaedics, Research Laboratory for Biomechanics and Implant Technology, University Medicine Rostock, 142 Doberaner Street, D-18057 Rostock, Germany
E-mail: annett.klinder@med.uni-rostock.de

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Introduction

Monocytes are a multifunctional fraction of peripheral blood mononuclear cells (PBMCs) (1). They originate from clonogenic progenitors in the bone marrow (BM), the so called macrophage dendritic cell precursors (MDPs) which give rise to common dendritic cell (DC) precursor (CDP), a DC-restricted precursor in the BM, and cMOP (common monocyte progenitor), a monocyte-restricted BM precursor (2). The precursor cMOP in turn gives rise to monocytes. So far three distinct monocyte fractions have been identified: 'classical' $CD14^{++}CD16^{-}$, 'intermediate' $CD14^{++}CD16^{+}$ and 'non-classical' $CD14^{+}CD16^{+}$ monocytes which respective to their functional properties have also been described as 'phagocytic', 'inflammatory' and 'patrolling' (3). For a long time it was thought that adult BM derived monocytes migrate into different tissues, e.g., bone, spleen, the central nervous system, liver, lung and connective tissue as a reaction to local environmental conditions, and differentiate into macrophages (4,5). However, more recent research revealed that not only adult monocyte-derived macrophages but also embryonic macrophages and newborn monocyte-derived macrophages co-exist in adult tissue and function in the induction and regulation of immune responses and tissue healing as well as the regulation of homeostasis (2,5,6).

In orthopaedic research tissue-resident monocytes and macrophages play a central role with regard to the interaction between tissues and artificial materials. Not only are monocytes the first cells to attach to implant surfaces, they are also recruited to the tissue in case of inflammation and participate in the inflammation process either as monocytes directly or differentiated into macrophages. Additionally, when differentiating into osteoclasts they are involved in bone remodelling (7). However, since it is not feasible to isolate tissue-resident monocytes and macrophages to re-enact the interactions in tissue *in vivo*, the isolation and differentiation of BM derived monocytes from blood provides a viable alternative to obtain sufficient numbers of monocytes and macrophages for *in vitro* experiments.

Thus, the aim of the present *in vitro* study was to establish an *in vitro* system well-suited to culture human monocytic cells and to prevent adhesion-induced differentiation prior to the intended experimental set up. We analysed

the impact of different cell culture plastics materials and, additionally, cell culture media on the behavior of human mononuclear cells, especially on their adhesion behavior and differentiation into macrophages. Hence, peripheral blood mononuclear cells were cultivated on: i) low attachment; ii) cell-repellent and iii) temperature-sensitive culture plates in order to prevent adhesion-induced differentiation but allow cell proliferation. Thereby, we wanted to achieve sufficient numbers of monocytes available for subsequent *in vitro* investigations, in which we would focus on the activation and differentiation into macrophages under differing conditions.

Materials and methods

Cell isolation. PBMCs were isolated from human buffy coats from blood donations, which were provided by the Institute of Transfusion Medicine, Rostock University Medical Center, anonymously (Local Ethics Committee: Registration number: A2011-140). PBMCs were extracted from prepared buffy coats by means of density gradient centrifugation (Ficoll Hypaque method) on lymphocyte separation medium in blood separation tubes (both from PAA Laboratories GmbH, Coelbe, Germany). The interphase containing lymphocytes and monocytes (density: 1.07 g/ml) was aspirated with a Pasteur pipette, washed twice in PBS and cells were cultivated in different culture plates (temperature sensitive plate, low-attachment plate, cell-repellent plate) using Roswell Park Memorial Institute medium (RPMI) 1640 (PAA Laboratories GmbH, Cölbe, Germany) containing 10% fetal calf serum (FCS), 1% penicillin/streptomycin (all from Gibco; Thermo Fisher Scientific, Inc., Dreieich, Germany) and 2% L-glutamine (PAA Laboratories GmbH), as well as in Dulbecco's modified Eagle's medium Nutrient Mixture F-12 (DMEM-F12) containing 10% FCS, 1% penicillin/streptomycin (all from Gibco; Thermo Fisher Scientific, Inc.) plus 0.5% L-ascorbic acid (50 μg/ml) for comparison. RPMI 1640 was developed for the culture of peripheral blood lymphocytes and is widely used for suspension culture of white blood cells while DMEM-F12 is a relatively rich and complex culture medium suitable for a wide variety of mammalian cells (8). Cells ($1x10^7$ cells/ 6-well in 3 ml) were incubated at 37˚C, 5% CO_2 and 21% O_2 for seven days.

Use of modified cell culture plates. Cell cultivation was accomplished in 6-well cell culture plates with different modifications of the plate surfaces in order to cultivate monocytes while preventing adhesion induced differentiation.

Nunc UpCell™ culture plates (Thermo Fisher Scientific, Inc.) exhibit a temperature-sensitive surface coating consisting of a covalently immobilized polymer poly (N-isopropylacrylamid) (PiPPAm) that is slightly hydrophobic at 37˚C and enables adherence and cell growth. When the temperature is reduced to less than 32˚C the coating evolves very hydrophilic characteristics and is able to bind water (9). At that point, adherent cells and the extracellular matrix detach from the Nunc UpCell™-surface, while viability, antigens and surface receptors are preserved. Thus, the use of enzymatic and mechanical methods for cell detachment can be avoided. TheNunc UpCell™-surface is therefore referred as 'temperature sensitive plate'.

The surface of Nunc HydroCell™ (Thermo Fisher Scientific, Inc.) culture plates is coated with a covalent immobilized and extremely hydrophilic polymer that averts cell attachment and prevents adhesion-dependent differentiation. It is called a 'low attachment plate' in the following.

Due to chemical modification of the applied plastics, Cellstar® Cell-Repellent Surface culture plates (Greiner Bio-One GmbH, Frickenhausen, Germany) offer a cell-repellent surface. According to the manufacturer's information cell adhesion is inhibited effectively and cultivation of tumor cells, embryonic cells and semi-adherent and adherent cells such as macrophages is enabled. The appellation 'cell-repellent plate' is used below.

For comparison standard tissue culture (TC) -treated polystyrene plates (Corning™ Falcon™ TC-treated multi-well plate; Thermo Fisher Scientific, Inc.) that support cell adhesion were used. TC-treatment involves vacuum gas plasma treatment to permanently modify the plate surface. The incorporation of negatively charged functional groups creates a hydrophilic surface well suited for cell attachment (10,11).

All cultivations were carried out under identical conditions at 37˚C, 5% CO_2 and 21% O_2 in a humidified atmosphere.

Fluorescence activated cell sorting (FACS) of monocytes/macrophages. Surface markers of monocytes and macrophages were analysed by staining with antibodies against CD14 [phycoerythricin (PE)-labeled] and CD68 [fluorescein isothiocyanate (FITC)-labeled] (eBioscience; Thermo Fisher Scientific, Inc.). Suspension cells were collected, centrifuged at 118 x g for 8 min, washed with PBS (PAA Laboratories GmbH) containing 1% bovine serum albumin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and centrifuged again before being counted. Adherent cells were washed twice with PBS, trypsinized, centrifuged at 118 x g for 8 min, re-suspended in in PBS/BSA (1%) and counted with a Thoma haemocytometer. The cell number was adjusted to $1x10^6$ cells/ml in PBS/BSA (1%). Staining of surface markers was performed by incubating 100 μl cell suspension with 0.1 μg of CD14 and CD68 antibodies for one hour in the dark. Appropriate isotype controls (PE-conjugated IgG1 and FITC-conjugated IgG2b; eBioscience; Thermo Fisher Scientific, Inc.) served as negative controls. After incubation the cells were washed three times in PBS/BSA (1%) in order to remove unbound antibodies. After re-suspension in PBS/BSA (1%). Flow cytometry was performed immediately on a FACS calibur flow cytometer (Becton; BD Biosciences, Franklin Lakes, NJ, USA).

Data were analysed with the FlowJo 10 program (Tree Star, Inc., Ashland, OR, USA). The fraction of cells considered as monocytes (MF) was gated by forward and side scatter. Fluorescence signals of the cell populations were integrated to a histogram with a specific isotype and an unstained control. The number of positive cells was gated against a background of no more than 5% of the isotype control. Results are expressed as percentage of positive cells with regard to total cells and to MF. Additionally, the ratio of the median fluorescence intensity (MFI) was calculated by dividing the MFI of the stained sample by the MFI of the unstained sample.

Cell biological tests. Cell viability was determined with the colorimetric WST-1 assay (Roche, Grenzach-Wyhlen, Germany) as well as live/dead staining (Invitrogen; Thermo Fisher Scientific, Inc). A cytokine multiplex [IL6, IL8 and

Monocyte Chemoattractant Protein-1 (MCP-1)] (Bio-Rad Laboratories GmbH, München, Germany) was performed to assess immune-stimulatory effects of the plate surfaces. All assays were conducted according to the manufacturer's instructions.

For the assessment of proliferation, PBMCs were seeded into 6-well plates (either TCPs or cell-repellent plates) at a concentration of $1x10^7$ cells per well. Cells were harvested after 24 h, 3 days and 7 days and the number of adherent cells as well as cells in suspension was counted using a Thoma haemocytometer. Viable and dead cells were differentiated by Trypan Blue staining.

Statistical analysis. Statistical analysis was performed by GraphPadPRISM v.7.0 (GraphPad Inc., San Diego, CA, USA). As the number of replicates was small, parametric tests were used for analyses with only three replicates. Normality was assessed using the Shapiro-Wilk test when the number of values was ≥4. Normally distributed data were analysed using parametric tests, while non-parametric tests were applied if data were not normally distributed. The individual tests performed for the results are indicated in the text. All P-values resulted from two-sided statistical tests and $P<0.05$ was considered to indicate a statistically significant difference.

Results

CD14 and CD68 expression on PBMCs cultured in different culture plates. In order to avoid attachment of blood derived monocytes and subsequent differentiation into macrophages PBMCs from buffy coats were cultured for seven days on three different brands of tissue culture plates that were designed to prevent cell attachment. A normal TC-treated polystyrene plate which encourages cell attachment was used as a control. Adherent and suspension cells were analysed by flow cytometry for the surface expression of the monocyte and macrophage markers CD14 and CD68, respectively (3,5,12). Additionally, forward and side scattering was used to gate the fraction of cells considered to be monocytes (13,14). Nearly all CD14 positive cells were detected in this gated fraction (Fig. 1A). As expected the percentage of the gated monocyte fraction (MF) was higher among adherent cells compared to suspension cells ($P<0.001$, Two-Way ANOVA, n≥4; Fig. 2). However, post hoc tests showed only significant differences in MF percentage between adherent and suspension cells when cells were cultured in RPMI in cell-repellent plates ($P=0.0027$, Tukey's multiple comparison test, n≥4) and temperature-sensitive plates ($P=0.0104$, Tukey's multiple comparison test, n=4). When analysing CD14 positive cells an influence of the cell culture medium was also apparent (Fig. 1B-E). There were no significant differences between adherent and suspension cells or between the different culture plates when cells were cultivated in DMEM F12, neither when evaluating percentage of CD14 positive cells per total cells (Fig. 3A), percentage of CD14 positive cells per MF or ratio of median fluorescence intensity (MFI) of CD14-stained to unstained cells. In contrast, the percentage of CD14 positive adherent and suspension cells per total cells cultured with RPMI medium was significantly higher in cell-repellent plates compared to the control plates (Kruskal-Wallis test with Dunn's multiple comparisons test as post test, $P=0.0431$ and $P=0.0389$ for adherent and suspension cells, respectively, n ≥4, Fig. 3B). On all culture plates there was, in general, only low expression of surface CD68 with negligible differences, pointing to less differentiated macrophages (Figs. 4 and 5).

Since differences were only observed between cells cultured with RPMI in cell-repellent plates and in control plates, subsequent tests were performed under these conditions only.

Proliferation of adherent and suspension cells derived from PBMCs in culture on cell-repellent and control culture plates. In order to test whether the increase in CD14 positive cells was due to proliferation, adherent and suspension cells were counted on day 1, 3 and 7 after seeding of PBMCs from four different donors into cell-repellent and normal TCPs. There was no increase in the number of cells. On the contrary, the number of cells in suspension decreased significantly over time (Repeated measures two-way ANOVA, effect for time $P<0.0001$) to approximately 75% of the initially seeded number of PBMCs. The decrease in suspensions cells is coherent with the facts that unstimulated PBMCs do not proliferate in culture (15,16) but are rather prone to apoptosis (17). However, the observed reduction in suspension cells was independent from the type of culture plate (Repeated measures two-way ANOVA, effect for plate $P=0.8411$, n=4; Fig. 6A).

Based on the hypothesis that the TC-treated culture plates (TCPs) encourage adhesion we expected higher numbers of adherent cells in the normal TCPs compared to the cell-repellent plates. While observations with light microscopy supported this hypothesis when counting the adherent cells only a significant difference was observed on day 3 with higher numbers of vital cells in normal TCPs compared to cell-repellent plates ($P=0.0433$, paired t-test, n=4; Fig. 6B).

Activation/differentiation of adherent and suspension cells from PBMCs in culture on cell-repellent and control culture plates. Activation and selection of CD14 positive cells may provide an alternative explanation for the observed increase. This could be due to the different surface chemistry. Indeed, different biomaterial surfaces not only influenced cell adhesion but modulated also cytokine and chemokine expression in monocytes and macrophages (18). We therefore analysed the cytokine IL6 and the chemokines IL8 and MCP1, which are associated with monocyte activation (19). MCP1 plays an essential in the recruitment of classical and inflammatory monocytes into inflamed tissue and these cells mature into inflammatory M1 macrophages which in turn secret the pro-inflammatory cytokine IL6 (3). The concentrations of IL8 and IL6 were on average approximately five times higher when PBMCs were cultivated in cell-repellent compared to normal TCPs (Fig. 7A and B). However, due to high inter-individual variation between the donors the changes only reached significance for IL8 (paired t-test, $P=0.0186$ and $P=0.3902$ for IL8 and IL6, respectively, n=3). There was no difference in the concentration of MCP1 between the plates after 7 days cultivation (paired t-test $P=0.5034$, n=3; Fig. 7C).

Additionally, the greater abundance of monocyte marker CD14 expressing cells resulted in higher numbers of adherent cells as measured by increased metabolic activity in subsequent cultivation experiments. In these experiments, PBMCs

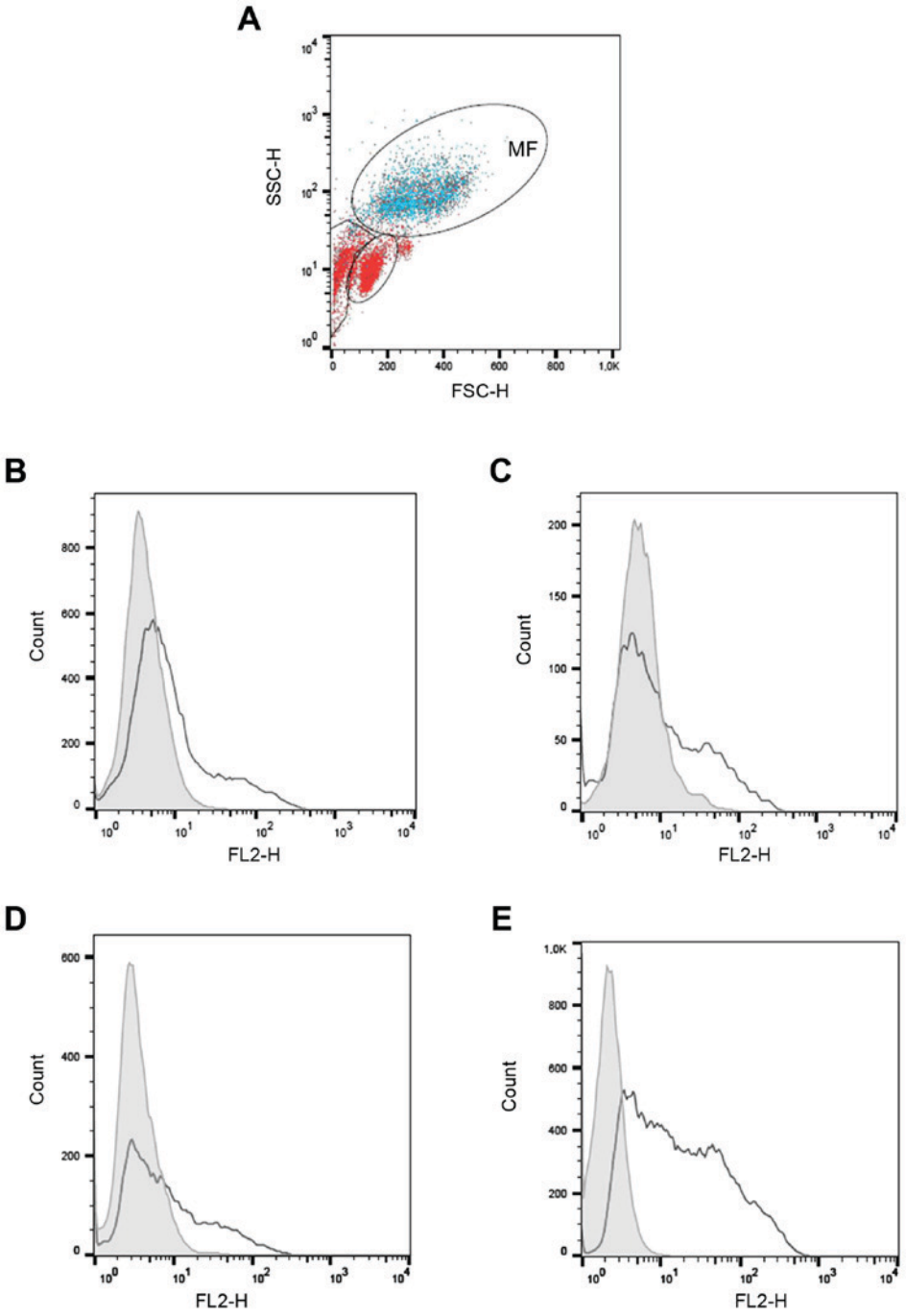

Figure 1. (A-E) Representative depiction of CD14 staining. (A) FSC and SSC gated MF overlaid with CD14 staining shown as blue dots (donor 14). Examples of comparison of CD14 stained cells in different cell culture media DMEM F12 (B and D) and RPMI (C and E) between TCPS control plates (B and C) and Repellent plates (D and E) analysed in the gated MF. Stained cells are shown as dark grey line against the background of the light grey shaded isotype control (all suspension cells, donor 14). Measurement was carried out with the BD FACS Calibur Flow Cytometer and data were analysed with the FlowJo 10 program. CD, cluster of differentiation; FSC, forward scatter; SSC, sideward scatter; MF, monocyte fraction; DMEM F12, Dulbecco's modified Eagle's medium nutrient mixture F-12; RPMI, Roswell Park Memorial Institute Medium; TCPS, tissue culture plates; BD, Becton Dickinson; FACS, fluorescence-activated cell sorting.

were initially cultivated in cell-repellent and normal TCPs. The cells in suspension were then transferred to control TCPs for another 72 h. Cells originating from the cell-repellent plate showed significantly increased metabolic activity compared to cells originally from the normal TC plate (paired t-test P=0.0008, n=3, Fig. 8A). This was probably due to an increased rate of adhesion of the cells originating from the cell-repellent plate as live/dead-staining of the adherent cells indicated higher cell numbers after re-seeding cells from cell-repellent onto normal TC plates. Fig. 8B displays the macroscopic cellular adhesion behavior after transfer from a cell-repellent plate to a TCPs surface.

Discussion

In standard *in vitro* cell cultivation procedures, tissue culture polystyrene dishes are used. Monocytes easily attach to these culture dishes, and immediately become activated. Thereby,

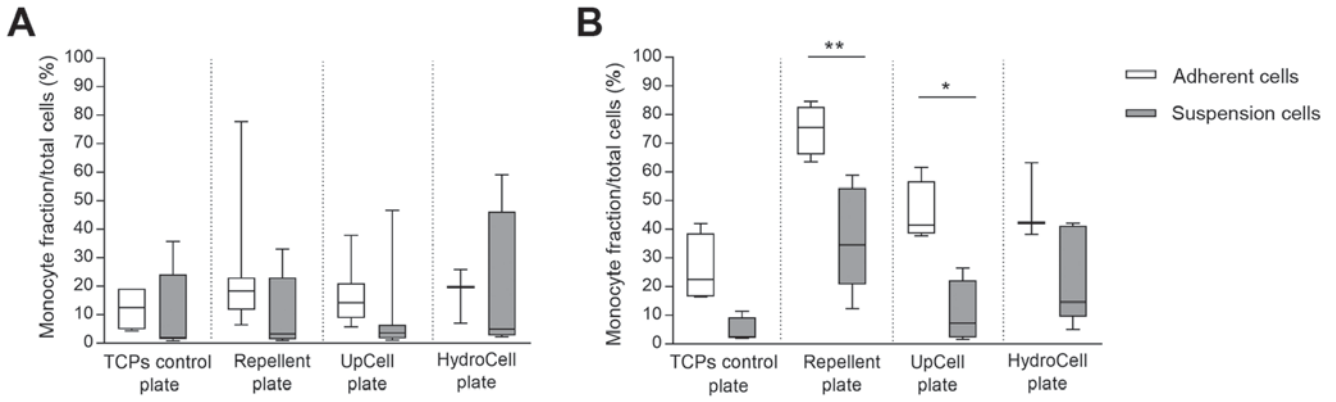

Figure 2. Percentage of cells in the monocyte fraction as gated with FSC and SSC after cultivation in different cell culture plates with (A) DMEM F12 or (B) RPMI. Measurement was carried out with the BD FACS Calibur Flow Cytometer and data were analysed with the FlowJo 10 program. Values are depicted as box plots with minimum, 25th percentile, median, 75th percentile and maximum of the gated cell number of the individual donors and experiments (n≥4). Statistical analysis was performed using Two-way ANOVA followed by Tukey's multiple comparisons as post test. *P<0.05, **P<0.01. FSC, forward scatter; SSC, sideward scatter; DMEM F12, Dulbecco's modified Eagle's medium nutrient mixture F-12; RPMI, Roswell Park Memorial Institute Medium; BD, Becton Dickinson; FACS, fluorescence-activated cell sorting, ANOVA, analysis of variance.

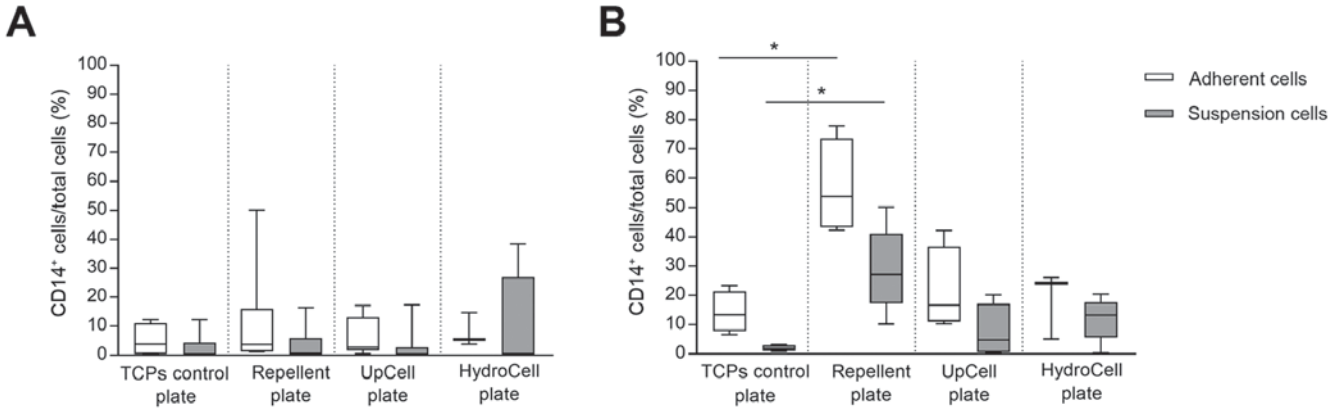

Figure 3. Percentage of CD14$^+$ cells per total cells as determined by flow cytometry after cultivation in different cell culture plates with (A) DMEM F12 or (B) RPMI. Cells (1x10^5) were stained with 0.1 μg of CD14 antibodies as well as the isotype control IgG1 and measured with the BD FACS Calibur Flow Cytometer. Data were analysed with the FlowJo 10 program and values are depicted as box plots with minimum, 25th percentile, median, 75th percentile and maximum of CD14 positive cells per total cells for the individual donors and experiments (n≥4). Statistical analysis was performed using Kruskal-Wallis test with Dunn's multiple comparisons test as post hoc test. *P<0.05. CD, cluster of differentiation; DMEM F12, Dulbecco's modified Eagle's medium nutrient mixture F-12; RPMI, Roswell Park Memorial Institute Medium; IgG1, immunoglobulin G1; BD, Becton Dickinson; FACS, fluorescence-activated cell sorting; TCPS, tissue culture plates.

they start to differentiate into macrophages which strongly adhere to TCPs because of the chemical and physical properties (20) and are difficult to detach using current enzymatic or mechanical methods. Surface functionality affects integrin-mediated cell adhesion (21) as well as cell activation and fusion (22). TCPs-promoted cell adhesion induces differentiation of non-adherent monocytes into adherent macrophages (23). Since cell differentiation is induced by the surface functionalities (24-26) cell cultivation in appropriate surface-modified culture plates could be an alternative to prevent this.

Our data indicate that the cultivation of PBMCs on cell-repellent culture plates favoured a monocytic phenotype as judged from the significantly increased CD14 expression. Low surface CD68 expression, especially in the adherent cells, was consistent with previous studies (12,27) and was associated with resting M0 macrophages while activation and differentiation to a M1 phenotype was characterised by increased surface CD68 expression (27). The increased number of CD14 positive cells after seven days cultivation in cell-repellent plates is probably rather due to a selection of a subgroup or an activation of certain cells as overall proliferation did not differ between the culture plates. This notion is supported by the observation, that seven-day but not three-day cultivation in cell-repellent plates led to elevated adherence of monocytes and improved survival of macrophages in subsequent experiments (data not shown), suggesting a differentiation of the cells over time. The elevated cytokine levels measured in cell-repellent plates corroborate the idea. This is in concordance to previous studies which showed that reduced adhesion coincided with an increase in cytokine production (18,28,29). Adhesion is mainly influenced by the surface characteristics of the used materials (25). Physio-chemical properties such as surface roughness, topography and ductility as well as surface charge and chemistry (functional groups like hydroxyl or carboxyl ones) either promote or limit the degree and type

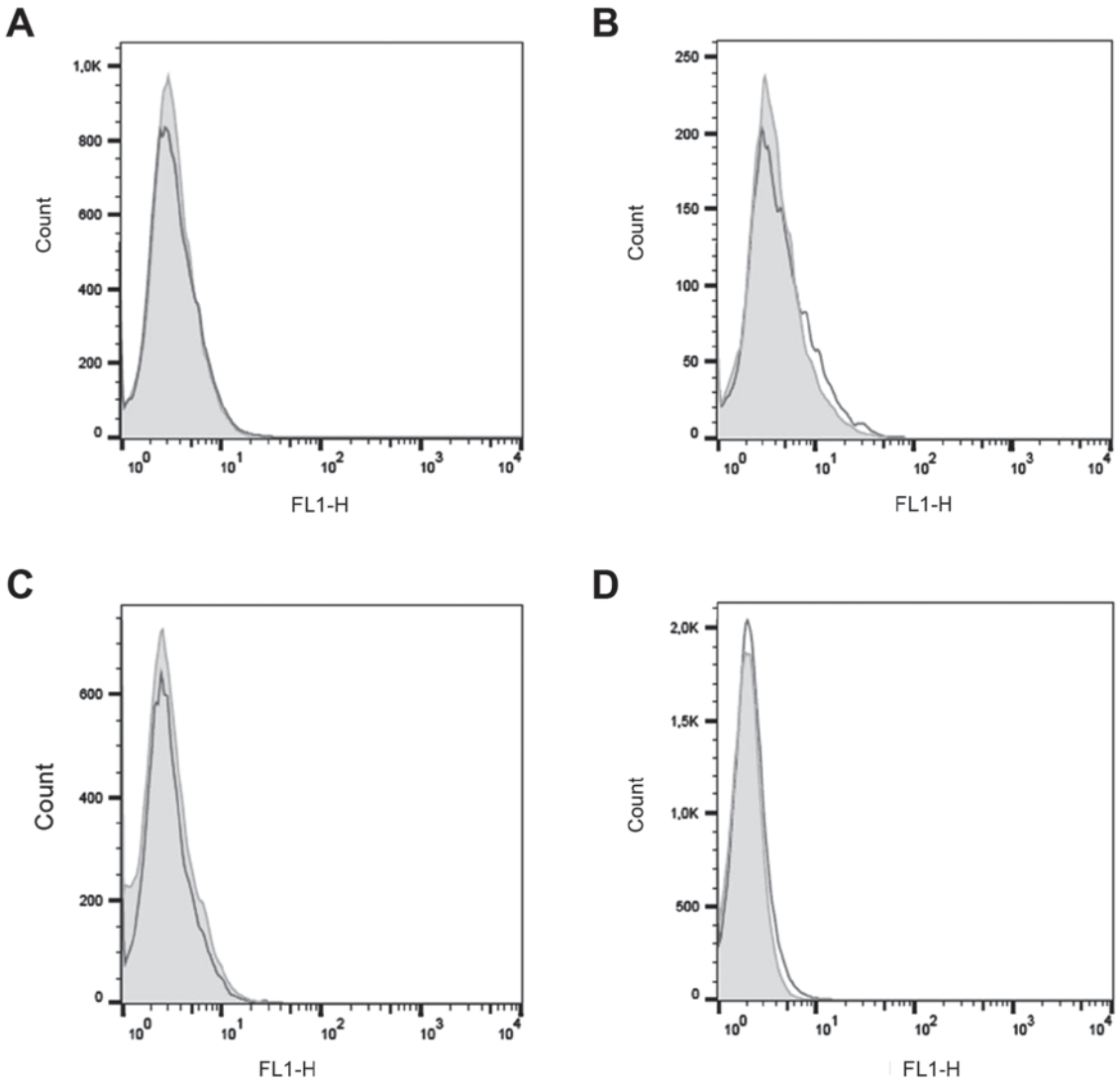

Figure 4. (A-D) CD68 stained cells. Comparisons of CD68-stained cells in different cell culture media [(A and C) DMEM F12 and (B and D) RPMI] between TCPS control plates (C and D) and Repellent plates (A and B) analysed in the gated MF. Stained cells are shown as dark grey line against the background of the light grey shaded isotype control (all suspension cells, donor 14). Measurement was carried out with the BD FACS Calibur Flow Cytometer and data were analysed with the FlowJo 10 program. CD, cluster of differentiation; DMEM F12, Dulbecco's modified Eagle's medium nutrient mixture F-12; RPMI, Roswell Park Memorial Institute Medium; TCPS, tissue culture plates; MF, monocyte fraction; BD, Becton Dickinson; FACS, fluorescence-activated cell sorting.

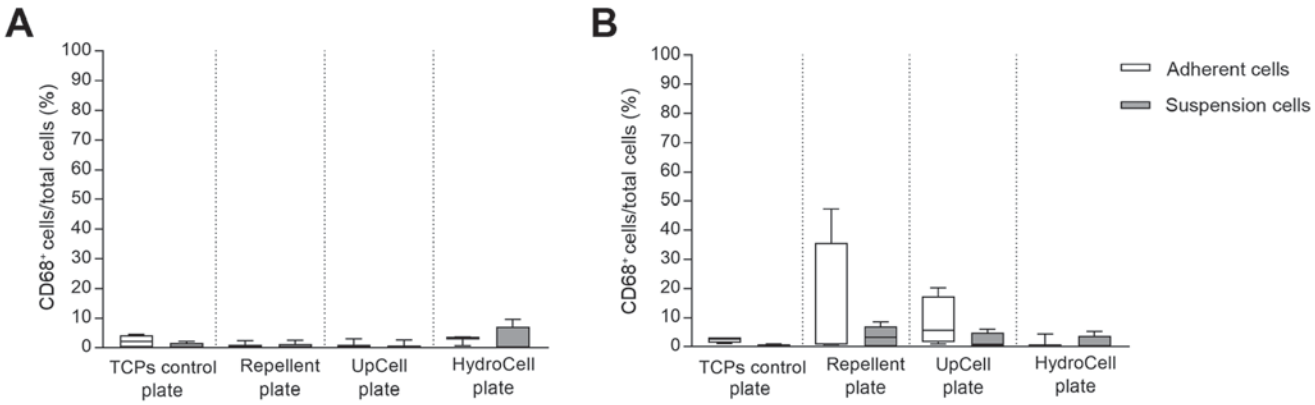

Figure 5. Percentage of $CD68^+$ cells per total cells as determined by flow cytometry after cultivation in different cell culture plates with (A) DMEM F12 or (B) RPMI. Cells ($1x10^5$) were stained with 0.1 μg of CD68 antibodies as well as the isotype control IgG2 and measured with the BD FACS Calibur Flow Cytometer. Data were analysed with the FlowJo 10 program and values are depicted as box plots with minimum, 25th percentile, median, 75th percentile and maximum of CD68 positive cells per total cells for the individual donors and experiments ($n \geq 4$). Statistical analysis was performed using Kruskal-Wallis test with Dunn's multiple comparisons test as post hoc test. CD, cluster of differentiation; DMEM F12, Dulbecco's modified Eagle's medium nutrient mixture F-12; RPMI, Roswell Park Memorial Institute Medium; IgG2, immunoglobulin G2; BD, Becton Dickinson; FACS, fluorescence-activated cell sorting; TCPS, tissue culture plates.

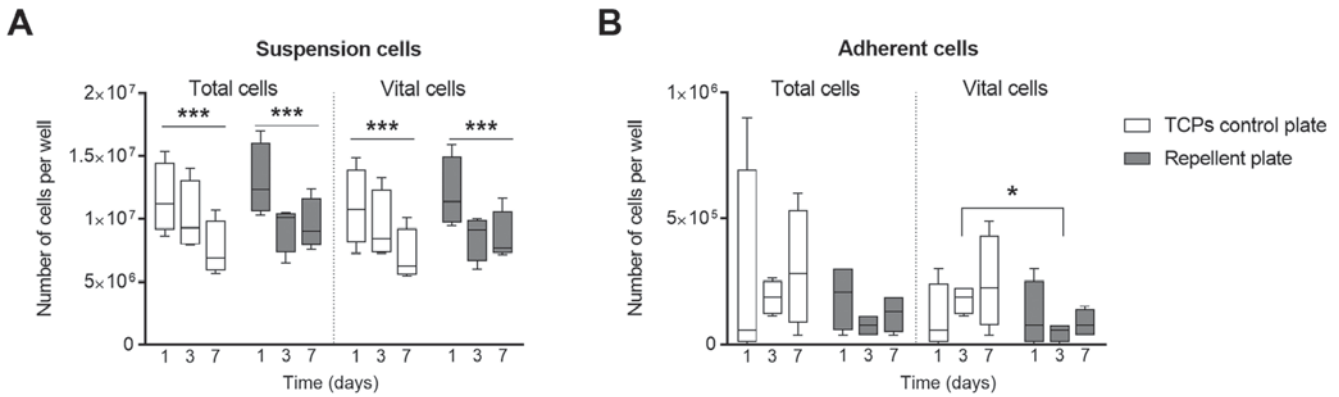

Figure 6. Cell number of (A) suspension and (B) adherent cells (PBMCs) after 1, 3 and 7 days of cultivation in Repellent and TCPS control plates with RPMI. Freshly isolated PBMCs were seeded at a concentration of $1x10^7$ cells per well and harvested after 24 h, 3 days and 7 days. The number of cells was counted using a Thoma haemocytometer and Trypan Blue staining. Values are depicted as box plots with minimum, 25th percentile, median, 75th percentile and maximum of numbers of PBMCs from four different donors. Statistical analysis was performed using Repeated measures two-way ANOVA. *P<0.05; ***P<0.0001. PBMCs, peripheral blood mononuclear cells; TCPS, tissue culture plates; RPMI, Roswell Park Memorial Institute Medium.

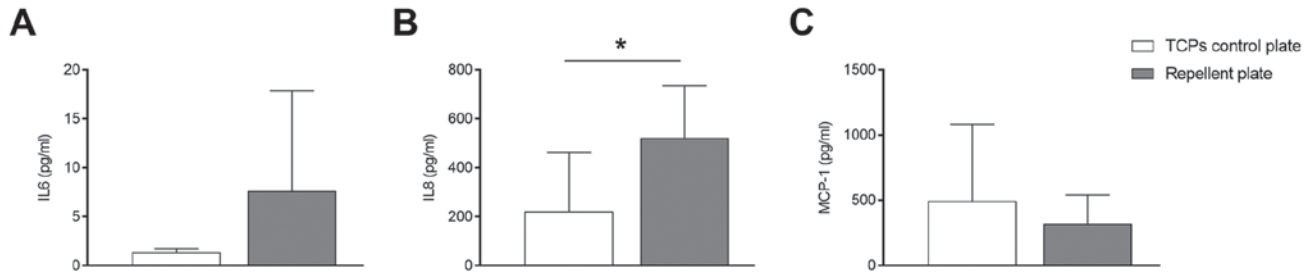

Figure 7. Protein concentrations of (A) IL-6, (B) IL-8 and (C) MCP-1 in the supernatant of PBMCs after 7 days of cultivation in in Repellent and TCPS control plates with RPMI. Values are depicted as mean ± SD of three different donors. Statistical analysis was performed using either paired t-test. *P<0.05. IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TCPS, tissue culture plates; RPMI, Roswell Park Memorial Institute Medium.

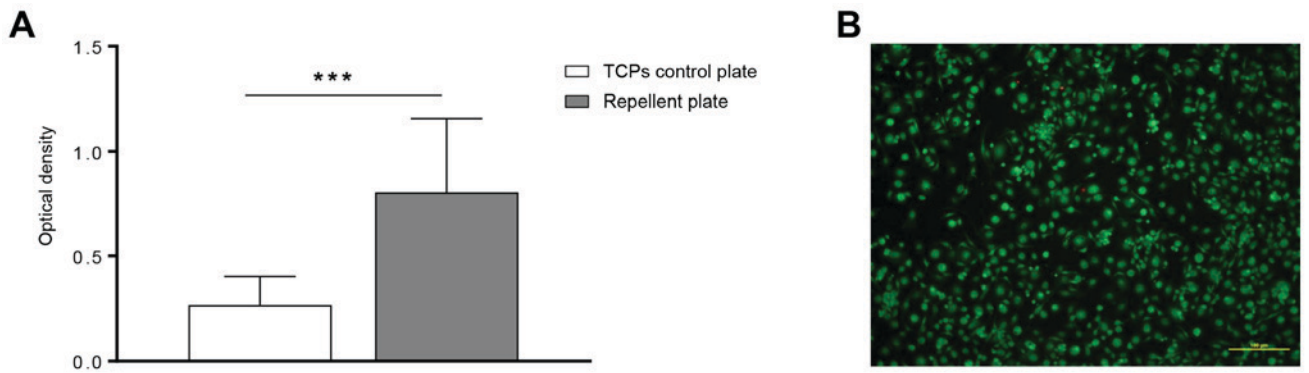

Figure 8. Comparison of metabolic activity of cells between repellent and control TCPS after transfer into normal TCPs as measured by (A) WST-1 assay and (B) live/dead staining of cells after transfer from cell-repellent culture plates into normal TCPs. Scale bar, 100 μm. Values are depicted as mean ± SD of three different donors. Statistical analysis was performed using paired t-test. ***P<0.001. TCPS, tissue culture plates; WST-1, water-soluble tetrazolium salt 1.

of protein adsorption and hence cell adhesion (24,30-35). The process is determined by cell-adhesion-mediating molecules (e.g., vitronectin, fibronectin) and their corresponding receptors (integrins). The resulting strength of adhesion regulates cell differentiation, proliferation and activity (32,34,36). Some studies point out a high adhesion potential for hydrophilic surfaces and a decreased potential for hydrophobic ones for monocytes and macrophages (24,32,35), while others mention an opposite influence (28,31,33,37) or cell apoptosis by hydrophilic surfaces (38). Moreover, monocyte adhesion depends on specific properties of hydrophilic surfaces; for example, reduced carbon content and increased oxygen and nitrogen presence (20) as well as their impact on binding of extracellular matrix proteins. Thus, surface charge and functional groups are the crucial factors for the strength of binding. Additionally, cell adhesion to either hydrophobic or hydrophilic surfaces seem to be dependent on serum content of the used culture medium as proteins present in FCS can influence monocyte adhesion. Some serum proteins such as fibronectin and immunoglobulins seem to promote adhesion while others

like von Willebrand factor and albumin are thought to inhibit long-term monocyte adhesion (35). The combination of all these factors might explain the different results observed for cultivation of PBMCs in the tested culture plates.

Based on our results cell cultivation with RPMI in adhesion-inhibiting culture plates may present an alternative to increase the fraction of monocytes yielded from PBMCs. While cultivation in cell-repellent plates induced the production of certain cytokines, the effect was still small and subsequent experiments showed that cells transferred from cell-repellent into normal TC-treated culture plates could still be further activated by metal ions, particles or bulk materials in order to mimic *in vivo* situations such as aseptic loosening or sufficient integration of implants (39-41).

In conclusion, our methodology of using the Cellstar® Cell culture plate with Cell-Repellent Surface combined with RPMI medium is an effective approach to selectively cultivate monocytes by providing inhibition of cell attachment. Improved adhesion and differentiation into macrophages occurred after transfer onto a cell-adherent TCPs surface. Thereby, we were able to increase the number of monocytes for subsequent *in vitro* investigations focusing on the activation and differentiation into macrophages under different conditions.

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Availability of data and material

All data generated or analysed during the present study are included in this published article.

Authors' contributions

AK planned and performed the growth experiments, statistically analysed all flow cytometry and cell biology data and drafted the manuscript. JM designed the study, performed the cytokine ELISAs and live/dead staining, analysed the data and was a major contributor in writing the manuscript. AJH planned and designed the study and was a major contributor in writing the manuscript.PS and AS performed the flow cytometry experiments, analysed these data and contributed to the writing. RB planned the study and was involved in the design of the experiments and revised the manuscript.

Ethics approval and consent to participate

PBMCs were isolated from human buffy coats from blood donations which were provided by the Institute of Transfusion Medicine, University Medical Center Rostock anonymously (Local Ethics Committee: Registration number: A2011-140). Only blood from those volunteers who consented to the use of their donation for research purposes was used.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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