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PAPER

Inflammatory mimetic microfluidic chip by immobilization of cell adhesion molecules for T cell adhesion†

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Leukocyte adhesion to adhesion molecules on endothelial cells is important in immune function, cancer metastasis and inflammation. This cell–cell binding is mediated *via* cell adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) found on endothelial cells. Because these adhesion molecules on endothelial cells vary significantly across several disease conditions such as autoimmune diseases, inflammation or cancer metastasis, investigations of therapeutic agents that down-regulate leukocyte–endothelial interactions have been based on *in vitro* models using endothelial cell lines. Here we report a new model, an inflammatory mimetic microfluidic chip, which emulates leukocyte binding to cell adhesion molecules (CAM) by controlling the types and ratio of adhesion molecules. In our model, E-selectin was essential for the synergic binding of Jurkat T cells. Immunosuppressive drugs, such as tacrolimus (FK506) and cyclosporine A (CsA), were used to inhibit T cell interactions under the physiologic model of T cell migration at a ratio of 5 : 4.3 : 3.9 (E-selectin : ICAM-1 : VCAM-1). Our results support the potential usefulness of the inflammatory mimetic microfluidic chip as a T cell adhesion assay tool with modified adhesion molecules for applications such as immunosuppressive drug screening. The inflammatory mimetic microfluidic chip can also be used as a biosensor in clinical diagnostics, drug efficacy tests and high throughput drug screening due to the dynamic monitoring capability of the microfluidic chip.

Introduction

Study about the leukocyte adhesion and migration in endothelium are important in autoimmune diseases, inflammation and cancer metastasis.[1,2] Interactions between T cells and endothelial cells are mediated by intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and selectins (E-selectin, P-selectin, and so on) expressed on endothelial cells.[3,4] Each adhesion molecule has an intrinsic function. E-selectin mediates T cell rolling, which is the initiation step in leukocyte migration. ICAM-1 and VCAM-1 promote firm binding of T cells, which induces the trans-migration step.[5–7] Adhesion molecules on endothelial cells vary significantly with several disease conditions, and T cell adhesion and migration are affected by these CAMs on endothelial cells. Thus, investigation of therapeutic agents that down-regulate leukocyte–endothelial interactions has focused on markedly regulating the progression of autoimmune responses in a number of *in vitro* models.[8–11]

One of the conventional adhesion assays is the T cell counting method on the endothelial cell monolayer in a static state.[12–14] This static method cannot induce shear stress, which initiates T cell rolling along the vessel surface, a precondition for firm binding on endothelial cells and trans-migration into the tissue.[15] Thus, this method is problematic when used to study leukocyte activation and expression of adhesion molecules.[16,17] Recent studies have used the continuous flow-based microfluidic cell adhesion assay, which mimics the human blood vessel by coating human umbilical vein endothelial cells (HUVECs) on the microfluidic chip.[18–20] This procedure, however, is laborious and time-consuming.[8,10,21–24] Moreover, controlling the density, type and ratio of the adhesion molecules by expression time makes studies on the intrinsic function of respective adhesion molecules or the influence of the respective functions of adhesion molecules difficult, and thus this assay is difficult to use as a reproducible drug screening system.

In this study, we developed a new inflammatory mimetic microfluidic chip method, which immobilizes adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 on the surface of the microfluidic chip. Because this system immobilizes the adhesion molecules on the chip surface instead of blood vessel

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cells, the densities, the types and ratios of the adhesion molecules can be optimized, suggesting the following advantages over cell-based drug screening systems: (i) convenience in the modification of density, type and ratio of adhesion molecules; (ii) no need to induce adhesion molecule expression, which can be time-consuming and laborious; (iii) higher stability; (iv) simple integration with surface plasmon resonance (SPR); and (v) suitability for high-throughput screening (HTS).[23,25–28] This novel inflammatory mimetic microfluidic chip may be useful as a drug screening tool to select targets and to verify their effectiveness for autoimmune diseases.[29,30] Furthermore, SPR as a monitoring system can make this microfluidic system powerful and suitable as a micro-total analysis system (μ-TAS) and clinical diagnostics, especially for monitoring the T cell binding of human primary T cells from autoimmune disease patients.

Materials and methods

Fabrication of the microfluidic chip

SU-8 2050 negative photoresist (MicroChem Corp, MA, USA) were spin-coated on silicon wafers at 1000–4000 rpm to fabricate channels 10, 40, 75 and 165 μm-wide. The transparent mask on the SU-8 coated wafers was used to pattern a channel structure under 365 nm of UV light. Polydimethylsiloxane (PDMS) (Dow Corning, MI, USA) was mixed at a 10 : 1 (w/w) ratio with a cross-linker and then poured onto the substrate with a microfluidic channel structure. The air bubble was removed with a vacuum desiccator and PDMS was cured at 80 °C for 2 hours. The PDMS channels were peeled from the substrate and punctured with a blunt-end punch to make inlets and outlets. To fabricate the microfluidic channel, a cover glass was prepared using piranha cleaning solution (a mixed solution of H_2O_2 and H_2SO_4 at a 3 : 7 (v/v) ratio) in an ultrasonic cleaner (Bransonic, CT, USA). The cleaned cover glass was washed with ethanol and water and dried with N_2 gas. The PDMS structure and cover glass binding surface were treated in a plasma cleaner (Harrick Plasma, NY, USA) at 18 watts for 1 minute to facilitate strong binding. The microfluidic channels were 2 mm-wide, 2 cm-long and 10, 40, 75, or 165 μm-high. The heights of the channels were confirmed by a Zygo non-contact white light interferometer.

Immobilization of CAMs on the microfluidic chip

The surface of the slide glasses on the channel were coated with 5 μL of (3-mercaptopropyl)-trimethoxy-silane (MPTMS) (Sigma, MO, USA) in the oven at 120 °C for 30 minutes. The glass surface was modified with 2 mg mL^{-1} of nitrilotriacetic acid-maleimide (NTA-maleimide) (Dojindo, Kumamoto, Japan) at room temperature for 15 minutes. Blocking for non-specific binding was accomplished with 1 mg mL^{-1} maleimide-PEG (ID Biochem, Ulsan, Korea) in a humidified chamber at room temperature for 15 minutes. To immobilize the adhesion molecules (E-selectin, ICAM-1 and VCAM-1), which have an amino terminal His-tag, 12 mg mL^{-1} of nickel chloride (Sigma, MO, USA) solution was added to the humidity chamber at room temperature for 15 minutes. The His-tagged adhesion molecules (Prospec, Rehovot, Israel) were diluted to a concentration of 0–50 μM in PBS solution (PAA, MA, USA) and infused into the channel at a shear stress of 2 dyn cm^{-2} using a syringe pump (New Era Pump System, NY, USA) (Fig. 1B).[31] The adhesion molecules were incubated overnight at 4 °C to immobilize them onto the surface. The states of the endothelial cells were emulated by controlling the density, type, and ratio of adhesion molecules (Fig. 1C) with a total density up to 25 μM. The inflammatory mimetic microfluidic chip was designed to have many parallel channels in order to achieve simultaneous binding between multiple adhesion molecules and T cells. Activated or drug-treated Jurkat T cells were infused at an optimized shear stress (2 dyn cm^{-2}) and T cell interactions were measured based on the level of T cell binding (Fig. 1D).

Detection of adhesion molecules in microfluidic channels

The microfluidic channels were incubated for 1 hour at room temperature with 5 μg mL^{-1} of monoclonal anti-E-selectin-fluorescein (Abcam, Cambridge, UK) or monoclonal anti-ICAM-1-fluorescein (R&D Systems, MN, USA) or monoclonal anti-VCAM-1-fluorescein (R&D Systems, MN, USA) to detect E-selectin, ICAM-1 or VCAM-1, immobilization on the surface, respectively. The entire solution was infused at 3 dyn cm^{-2} using a syringe pump (New Era Pump System, NY, USA). The channel was washed three times with PBS (3 dyn cm^{-2}). The fluorescence intensity of fluorescein-conjugated antibodies was observed by fluorescence microscopy (Olympus, Hamburg, Germany) and analyzed by analySIS LS Research (Olympus, Hamburg, Germany).

Jurkat T cell culture

Jurkat T cells were incubated in RPMI 1640 (Lonza, MD, USA) containing 10% fetal bovine serum (Hyclone, Logan, UT, USA), 1% L-glutamine and 1% penicillin–streptomycin (Lonza, MD, USA). Jurkat T cells were grown in tissue culture-treated polystyrene T-75 flasks (NUNC, Roskilde, Denmark) in a standard culture incubator with humidified air containing 5% CO_2 at 37 °C.

Optimization of the adhesion assay on the microfluidic chip

Jurkat T cells (2×10^6 cells) were activated with 10 ng mL^{-1} of phorbol myristate acetate (Sigma, MO, USA) and 1 μM ionomycin (Sigma, MO, USA) in complete media for 1 hour at 37 °C. All cells were labeled with 1 μM calcein-AM (Invitrogen, CA, USA) for 15 minutes at 37 °C. Microfluidic channels were prepared with E-selectin and activated Jurkat T cells (2×10^5 cells per channel) were collected and infused into the channels (10, 40, 75 and 165 μm high) at a shear stress of 0.8, 1, 2, 3, 5, and 10 dyn cm^{-2} to mimic physiologic T cell recruitment (1–6 dyn cm^{-2}) conditions,[15,32] followed by washing with PBS at the same shear stress (0.8, 1, 2, 3, 5, 10 dyn cm^{-2}) to remove unbound cells. Jurkat T cells adhered to adhesion molecules were observed based on calcein-AM fluorescence intensity using fluorescence microscopy (Olympus, Hamburg, Germany) and analyzed by analySIS LS Research (Olympus, Hamburg, Germany). Numbers of activated Jurkat T cells were counted

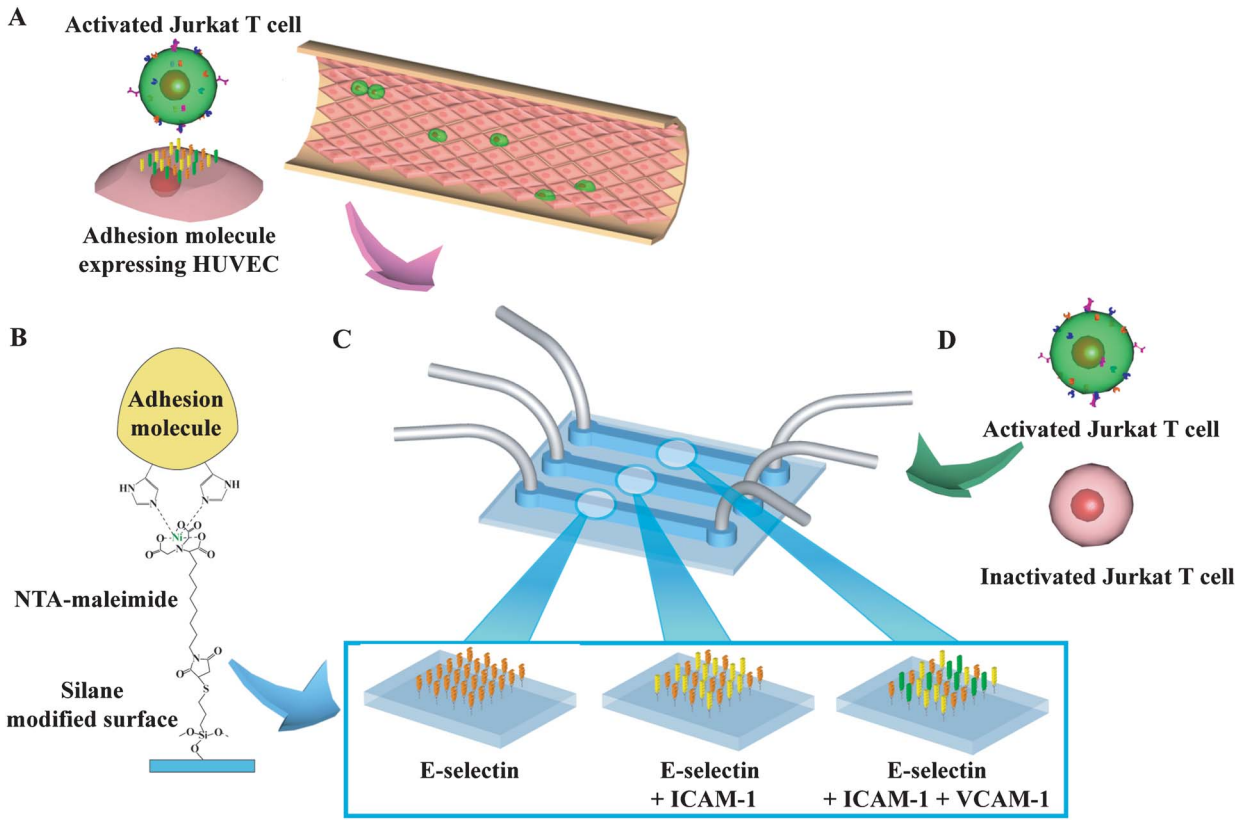

Fig. 1 Schematic of the inflammatory mimetic microfluidic chip. Microfluidic channels were treated with Jurkat T cells to assess the T cell interaction under disease conditions such as inflammation, cancer metastasis and autoimmune diseases. The microfluidic system mimics the binding of activated Jurkat T cells to the adhesion molecules expressed on HUVECs in blood vessels (A). To immobilize adhesion molecules such as E-selectin, ICAM-1 and VCAM-1, the microfluidic channel was treated with MPTMS and NTA-maleimide (B). The microfluidic system emulates the expression of adhesion molecules on HUVECs stimulated by mixtures of adhesion molecules (total density up to 25 μM) (C). Activated or drug-treated Jurkat T cells were infused at optimized shear stress (2 dyn cm^{-2}) and T cell interactions were measured based on the level of T cell binding (D).

four times at the respective microfluidic channels and the counts were converted into units per area.

Modification of adhesion molecules on the microfluidic chip

The following mixtures of adhesion molecules were prepared: E-selectin + ICAM-1, E-selectin + VCAM-1, and ICAM-1 + VCAM-1 mixtures. Each mixture was prepared at a 1 : 1 ratio to mimic the states of stimulated endothelial–leukocytes, which express the maximum level of adhesion molecules.[33,34] To compare the influence of interactions between adhesion molecules, T cell bindings on the mixture of adhesion molecules were performed as well as T cell bindings on respective adhesion molecules. The total concentration of respective adhesion molecules or adhesion molecule mixtures was adjusted to 25 μM. These mixtures were infused onto the channel surface, which were modified with MPTMS and NTA-maleimide. Jurkat T cells were activated with 10 ng mL^{-1} of PMA and 1 μM ionomycin.[35] Activated Jurkat T cells (2×10^5 cells per channel) were labeled with 1 μM calcein-AM at 37 °C for 15 minutes in complete media, collected and infused at 2 dyn cm^{-2} and then washed three times with PBS at the same rate (2 dyn cm^{-2}) to remove any unbound cells. The number of T cells bound on the adhesion molecule mixtures were compared with the number of T cells bound to respective adhesive molecules. A ratio of 1.0 indicated that the binding level of the mixture was the same as the sum of the separately detected binding levels on individual adhesion molecules.

Immunosuppressive drug screening

Microfluidic channels were treated with 5 : 4.3 : 3.9 mixtures of E-selectin, ICAM-1 and VCAM-1, which mimicked the state of adhesion molecule expression of stimulated endothelial–leukocytes after 4 hours of treatment with TNF-α and IL-1.[33,34] The total concentration of the adhesion molecules added up to 25 μM. The mixture solution was infused onto the channel surface, which was modified with MPTMS and NTA-maleimide. Jurkat T cells (2×10^6 cells) were activated with 10 ng mL^{-1} of PMA and 1 μM ionomycin at 37 °C for 1 hour in complete media and labeled with 1 μM calcein-AM at 37 °C for 15 minutes. Subsequently, the cells were treated with 10 ng mL^{-1} of PMA, 1 μM ionomycin, 10 nM tacrolimus (Sigma, MO, USA) and 1 μg mL^{-1} of cyclosporine A (Sigma, MO, USA) at 37 °C for 1 hour to suppress T cell activation.[36] The same number (2×10^5 cells) of activated and inactivated Jurkat T cells were collected and infused at an optimized shear stress of 2 dyn cm^{-2}, followed by washing with PBS (2 dyn cm^{-2}) to remove unbound cells. Jurkat T cells bound to the channel

surface were observed with fluorescence microscopy and counted four times in the respective microfluidic channels and the counts were converted into units per area.

Results and discussion

Jurkat T cell binding to adhesion molecules

Immobilized adhesion molecules were detected in the microfluidic channels with 5 μg mL^{-1} of FITC conjugated to an anti-E-selectin-antibody (ESI†), anti-ICAM-1-antibody and anti-VCAM-1-antibody (data not shown). After confirming the immobilization of adhesion molecules in the microfluidic channels, T cell interaction with the adhesion molecules was assessed based on the binding of activated Jurkat T cells to the E-selectin immobilized microfluidic channel as a proof of concept. Activated Jurkat T cells expressed sialyl-Lewis^x (sLe^x), lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-1 (VLA-1), which interact with the adhesion molecules, E-selectin, ICAM-1 and VCAM-1, respectively.[33,34] To determine the suitability of the inflammatory mimetic microfluidic chip as a T cell adhesion assay tool, the binding of activated Jurkat T cells to E-selectin was measured. E-selectin, which mediates the early stage of T cell binding, was immobilized inside the channel to mimic physiological conditions.[37]

Activated or inactivated Jurkat T cells were infused into the microfluidic channels, which were immobilized with or without E-selectin, in order to verify T cell binding to E-selectin. Activated Jurkat T cells without E-selectin (Fig. 2A) and inactivated Jurkat T cells on E-selectin (Fig. 2B) did not exhibit Jurkat T cell binding. Activated Jurkat T cells on E-selectin (Fig. 2C) indicated that activated Jurkat T cells were bound to the adhesion molecule, E-selectin, on the channel surface as expected. E-selectin is known to play a role in the initiation of T cell migration, and it is assumed that interaction with sLe^x makes T cell rolling slow enough to facilitate binding onto the channel surface.

Optimization of the inflammatory mimetic chip for T cell binding

The shear stress and channel geometry were optimized simultaneously to assess T cell binding at various shear stresses (0.8–10 dyn cm^{-2}) by infusing fluid and using various channel heights (10, 40, 75 and 165 μm) (Fig. 3A). The shear stress was calculated using the equation, $Q = (\tau_w wh^2)/6\eta$, where Q is the flow rate, τ_w is a wall shear stress, w is the width of the channel (2 mm), h is the height of the channel (10, 40, 75, 165 μm), and η is the viscosity of buffers at 37 °C (0.7×10^{-3} cp).[38] A range of shear stresses were determined that were slightly wider than the physiological shear stress of T cell recruitment (1–6 dyn cm^{-2}). The most binding (8.62 ± 1.02 cells mm^{-2}) was detected when activated Jurkat T cells were infused at 2 dyn cm^{-2}, as suggested by recent studies of cell-based microfluidic systems.[15,19] Trends of a bell-shaped curve that pivots around 2 dyn cm^{-2} were constantly observed for the channels 10, 40, 75, and 165 μm in height. Moreover, T cell binding was highest in the 40 μm-high microfluidic channels. Because the other conditions such as the density of the adhesion molecule and the shear stress of the infusing fluid had been optimized, we assumed that the low T cell binding in 75 and 165 μm-high channels was due to a decreased chance of contact. T cell binding in the 10 μm channel was not observed because the activated Jurkat T cells were not detected inside the channel (data not shown), possibly because the 10–20 μm diameter T cells were stuck at the small channel entrance.

Fig. 2 Jurkat T cell binding to adhesion molecules. T cell adhesion assays were performed to assess the interaction of T cells with the adhesion molecule, E-selectin. Activated Jurkat T cells without E-selectin immobilized in a channel (A), inactivated Jurkat T cells with E-selectin immobilized in a channel (B), and activated Jurkat T cells with E-selectin immobilized on the surface (C). Fluorescence image of T cell binding and T cell interaction with E-selectin (insertions). All Jurkat T cells were labeled with calcein-AM and channels were treated with 25 μM E-selectin to immobilize. The scale bar is 200 μm.

Because the inflammatory mimetic microfluidic chip was designed to study the interactions of Jurkat T cells and adhesion molecules immobilized on the microfluidic bottom surface, determining the coating efficiency of adhesion molecules was a prerequisite step to determining the suitability of the microfluidic system. The conventional methods based on the detection of fluorescence conjugated antibody are not reliable enough for the evaluation of immobilized molecules quantitatively; thus a Jurkat T cell adhesion assay was performed to identify the influence of adhesion molecule density. E-selectin, which mediates the early step of T cell binding,[39] was determined to be the most effective immobilizer and was perfused at concentrations of 0, 6, 25, 50, 100 μM (Fig. 3B). T cell binding was saturated (3.95 ± 0.14 cells mm^{-2}) over 25 μM. The results suggest a significant influence of adhesion molecule density, shear stress and channel geometry on T cell binding, and we were able to determine optimized conditions for the T cell adhesion assays.

The effect of adhesion molecules on T cell binding

Representative adhesion molecules are known to show different binding strengths during T cell migration. E-selectin, for example, is known to mediate T cell rolling with a weak binding strength and ICAM-1 is known to meditate strong binding. To identify the different roles of E-selectin, ICAM-1, and VCAM-1, we verified the different binding strengths *via* the T cell adhesion assay on an inflammatory mimetic microfluidic chip. Contrary to the cell-based study, the inflammatory mimetic microfluidic chip is suitable for the study of T cell binding on each adhesion molecule respectively. To identify the differences in binding characteristics, each microfluidic channel was prepared with a single type of adhesion molecule, E-selectin, ICAM-1 or VCAM-1, and the same number of activated Jurkat T cells (2×10^5 cells) were infused into the channel (Fig. 4). The level of T cell binding to E-selectin was the lowest compared to the levels of T cell binding to ICAM-1 and VCAM-1, which mediates firm binding at 2 dyn cm^{-2} shear stress. This result indicates that the intrinsic

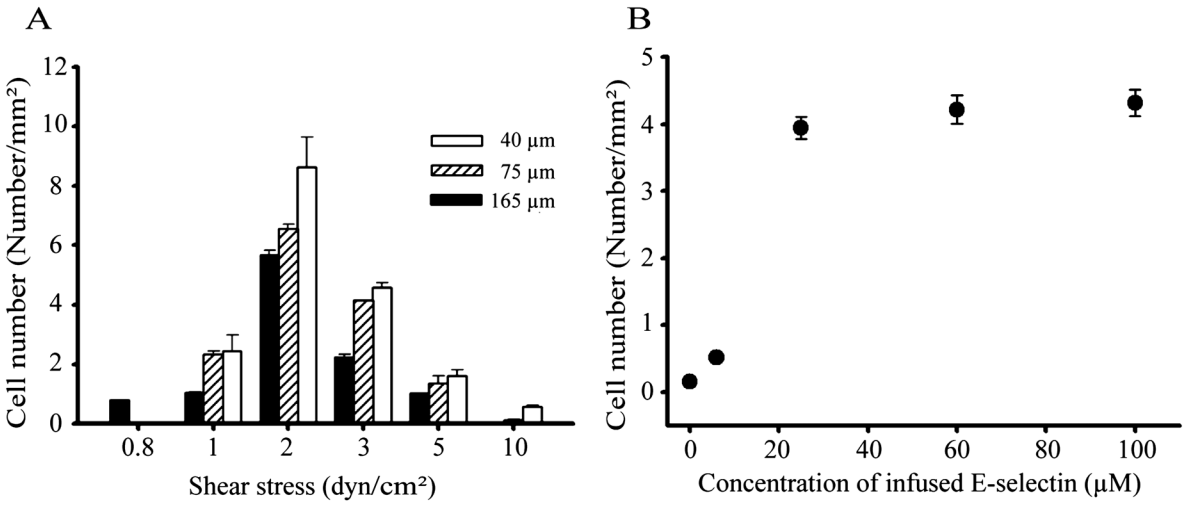

Fig. 3 Optimization of the inflammatory mimetic chip for T cell binding. Activated Jurkat T cells were infused into microfluidic channels, which were immobilized with E-selectin, in order to determine the effect of shear stress and channel height (A). Jurkat T cells were activated with PMA and ionomycin and infused with a shear stress of 0.8–10 dyn cm^{-2} to mimic the physiological shear stress of T cell recruitment (1–6 dyn cm^{-2}). Activated Jurkat T cells were perfused into 10, 40, 75 and 165 μm-high channels, the curve of which pivots around 2 dyn cm^{-2}. T cell binding in the 10 μm channel was not observed. Jurkat T cell binding on the channel was not detected at 75 μm and 165 μm at 0.8 dyn cm^{-2}, hence the corresponding areas are left empty. The influence of adhesion molecule density on T cell interaction was assessed with T cell binding of E-selectin (B). E-selectin was immobilized on the NTA-maleimide surface of 165 μm high microfluidic channels. Activated Jurkat T cells were infused at a shear stress of 2 dyn cm^{-2} into the channel. Jurkat T cells were activated with PMA and ionomycin and labeled with calcein-AM. The level of T cell binding was saturated at a 25 μM density of E-selectin. The number of bound Jurkat T cells was counted four times in the respective microfluidic channels.

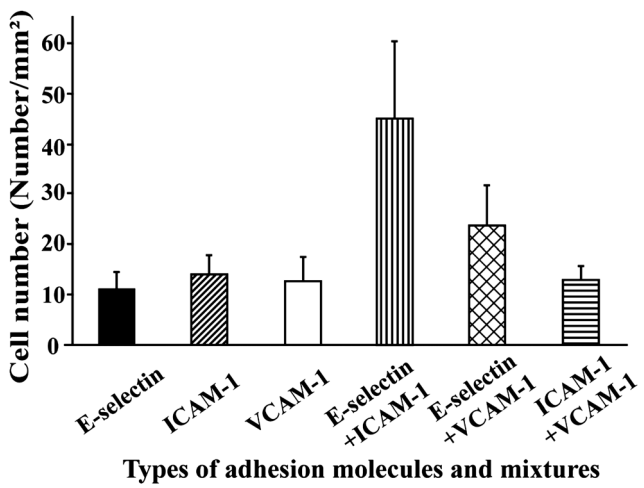

Fig. 4 T cell binding to respective adhesion molecules. T cell interaction was assessed by the number of Jurkat T cells bound to respective adhesion molecules, E-selectin, ICAM-1 and VCAM-1. To identify the interaction between adhesion molecules, adhesion molecule mixtures of E-selectin + ICAM-1, E-selectin + VCAM-1 and ICAM-1 + VCAM-1 (1 : 1 ratio) were compared. The collaborative influence between adhesion molecules was estimated based on the number of bound T cells in the adhesion molecule mixtures, which was divided by the sum of individual T cells bound to the respective adhesion molecules. Activated Jurkat T cells were infused (2 dyn cm^{-2}) into microfluidic channels (40 μm height) and detected with calcein-AM fluorescence. Each adhesion molecule (E-selectin, ICAM-1 and VCAM-1) was immobilized at a density of 25 μM. The density of adhesion molecule mixtures was also fixed at 25 μM to compare the combined binding effect with that of respective adhesion molecules. The number of bound Jurkat T cells was counted four times in the respective microfluidic channels and converted into units per area.

roles of adhesion molecules can be verified using the inflammatory mimetic microfluidic chip.

Under physiological conditions, each cell adhesion molecule works collaboratively by initiating T cell rolling along the vessel surface and then mediating firm binding to endothelial cells and trans-migration into the tissue. Thus, the collaborative influence of these respective adhesion molecules is also important for T cell binding and migration. We assessed Jurkat T cell binding on the inflammatory mimetic microfluidic chip, where the E-selectin + ICAM-1, E-selectin + VCAM-1 and ICAM-1 + VCAM-1 surfaces were coated with a 1 : 1 ratio of the respective molecules. We assumed the same ratio of coated molecules in microfluidic channel as ratio of adhesion molecule mixtures, because each His-tagged adhesion molecules were immobilized onto the NTA-maleimide surface, equivalently. We also assumed that the interaction of E-selectin with T cell, which initiates T cell migration, would affect the interactions of ICAM-1 and VCAM-1 with T cell, which mediate firm binding. To compare the collaborative influence of adhesion molecules, T cell bindings were assessed on respective adhesion molecule and adhesion molecule mixtures, both of which concentrations were 25 μM. The E-selectin + ICAM-1 mixture (1 : 1) had about 4 times more Jurkat T cell binding compared to individual E-selectin or ICAM-1 immobilized surface. This result indicates that the collaborative influence between E-selectin and ICAM-1 exists as expected. The E-selectin + VCAM-1 mixture (1 : 1) also showed about 2 times more Jurkat T cell binding compared to the binding on individual E-selectin or VCAM-1 immobilized surface. The T cell binding of the ICAM-1 + VCAM-1 mixture, however, was similar to the Jurkat T cell binding on individual E-selectin or ICAM-1 immobilized surface (Fig. 4). These results indicate that E-selectin is essential

for a collaborative influence in T cell binding. Also, the lower collaborative influence of the E-selectin + VCAM-1 mixture may be the result of the immune system evading effect of VCAM-1.[40] VCAM-1 is known to play conflicting roles, as it induces TNF-α/IL-1 expression of endothelial cells and enhances immune evasion of tumor cells.[34,40] The inflammatory mimetic microfluidic chip, which can modify the ratio of adhesion molecules, could be a powerful device for the investigation of therapeutic agents that down-regulate leukocyte–endothelial interactions.

Inflammatory mimetic microfluidic chip for immuno-suppressive drug screening

To verify the usefulness of the inflammatory mimetic microfluidic chip, the channel surface was modified with 5 : 4.3 : 3.9 mixtures (E-selectin, ICAM-1 and VCAM-1) to mimic the physiological expression states of endothelial cells after 4 hours of treatment with TNF-α and IL-1.[32,33] To verify the inhibition of T cell binding, activated Jurkat T cells were treated with immunosuppressive drugs, tacrolimus and cyclosporine A (Fig. 5A). As shown in Fig. 5B, an 11-fold decrease in T cell binding was observed in the immunosuppressive drugs treated Jurkat T cells in comparison to the non-treated cells (Fig. 5C). This result corresponded with the effect of the immune suppressors, which inhibit calcineurin signaling of the T cell activation process.[38,41] To apply the inflammatory mimetic microfluidic chip for advanced drug screening for inflammation, cancer metastasis or autoimmune diseases, further study of T cell interactions should be conducted under various states of physiological conditions.

Conclusions

An inflammatory mimetic microfluidic chip was created to measure T cell interactions with the immobilized cell adhesion molecules, E-selectin, ICAM-1, and VCAM-1. The density, type and ratio of cell adhesion molecules were modified on the surface of the microfluidic chip. E-selectin, which promotes T cell rolling, was essential for the synergic binding of Jurkat T cells. Moreover, the inhibition of T cell binding was verified after immunosuppressive drug treatment using this inflammatory mimetic microfluidic chip, which mimics the physiological conditions of T cell migration.

Although this microfluidic system is not a cell-based system, it can be used to mimic several disease conditions, such as autoimmune diseases, inflammation or cancer metastasis, by modifying the density, type and ratio of cell adhesion molecules. These model states of adhesion molecules can be used as an investigating device for the study of cell–cell interactions, and has the advantages of only requiring a small quantity of blood sample, low laboratory cost, and stability of storage as an advanced drug screening tool. By integration of this microfluidic system with a non-labeling and real-time monitoring system, such as SPR, this novel inflammatory mimetic microfluidic chip can create a powerful μ-TAS system, especially for monitoring the T cell binding of human primary T cells from autoimmune disease patients.

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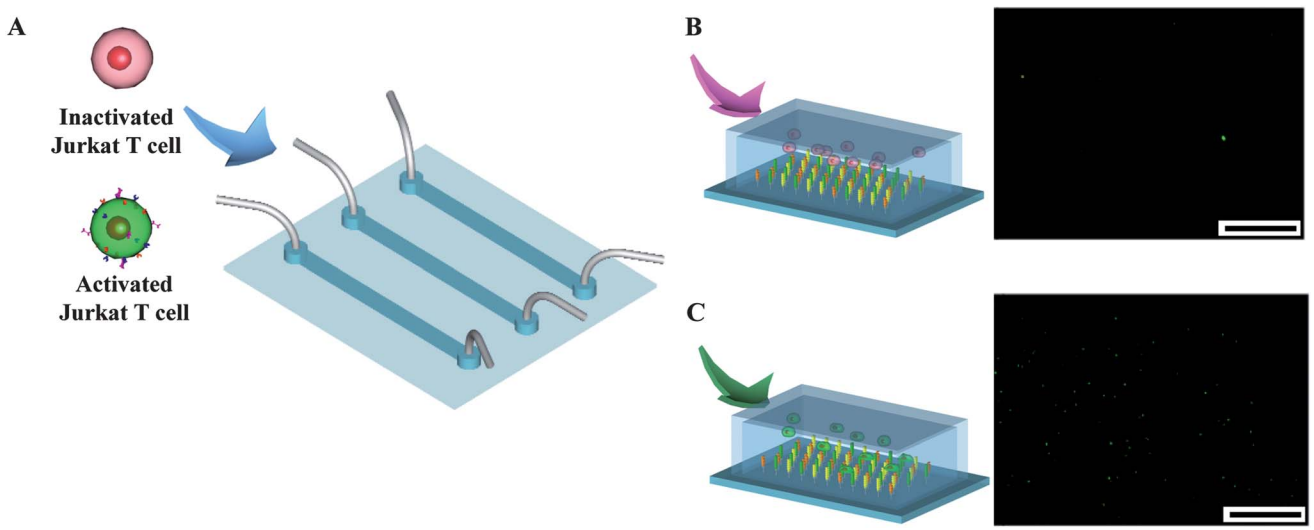

Fig. 5 Immunosuppressive drug screening using the inflammatory mimetic microfluidic chip. Immunosuppressive drug screening is one of the potential applications of the inflammatory mimetic microfluidic chip (A). Activated Jurkat T cells were infused into the channel, which mimicked the T cell migrating conditions during an inflammatory response. The E-selectin + ICAM-1 + VCAM-1 mixture was prepared to a ratio of 5 : 4.3 : 3.9, which emulated adhesion molecule expression, and was stimulated by TNF-α. To inactivate the Jurkat T cells, immunosuppressive drugs, 10 nM of tacrolimus (FK506) and 1 μg mL^{-1} of cyclosporine A (CsA), were perfused into the microfluidic channels (B). Activated Jurkat T cells without immunosuppressive drugs were perfused into the microfluidic channels as a control experiment (C). Jurkat T cells treated with immunosuppressive drugs showed a distinct decrease in T cell binding (B) compared to activated Jurkat T cells (C). To activate Jurkat T cells, 10 ng mL^{-1} of PMA and 1 μM of ionomycin were used, and activated Jurkat T cells were labeled with calcein-AM. The activated or drug-treated Jurkat T cells were perfused into the microfluidic channels at 2 dyn cm^{-2} of shear stress and washed with PBS three times (2 dyn cm^{-2}). The scale bar is 1 mm.

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