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Intercellular Mechanical Communication Drives Directional Migration of Jurkat T Immune Cells

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Abstract

While the regulatory mechanisms of chemokines in orchestrating adaptive and innate immune responses have been extensively elucidated, the contributions of biophysical mechanical cues to the modulation of immune cell migratory behaviors remain largely unexplored. In this study, we applied a modular co-culture experimental system, pairing suspension Jurkat T lymphocytes (with RAW 264.7 macrophages as a positive migratory control) with two distinct force-generating cell types—human airway smooth muscle cells (ASMCs) and human lung adenocarcinoma A549 cells—cultured on 1 mg/mL type I collagen hydrogels, to dissect the regulatory effects of intercellular mechanical signals on the directional migration of immune cells. We observed that Jurkat T cells exhibited robust, targeted directional migration toward force-generating cells on the 2D hydrogel surface, with migratory trajectories consistently oriented toward mechanically active target cells. Glutaraldehyde-mediated crosslinking of collagen matrix or higher-stiffness ~5 mg/mL Matrigel supplemented with 0.5 mg/mL collagen, which ablates cell-cell mechanical interactions, significantly impaired the directional attraction of immune cells, confirming the role of mechanotaxis in this migratory phenotype. Notably, Jurkat T cell migratory efficiency depended on the identity of force-generating cells, with enhanced directional migration toward ASMCs relative to A549 cells, a phenotype likely driven by cell type-specific intrinsic mechanical properties. Furthermore, 3D encapsulation within bulk collagen hydrogels significantly attenuated Jurkat T cell directional migration toward both force-generating cell types, relative to the 2D hydrogel platform. Collectively, our data demonstrate that T cell mechanotaxis is dually modulated by the intrinsic mechanical phenotype of force-generating cells and the dimensionality of the extracellular matrix microenvironment. These findings establish that biophysical mechanical cues, independent of and complementary to canonical chemokine gradients, are potent regulators of directional T cell migration, providing novel mechanistic insights into the emerging field of immunomechanics.

Keywords: immunomechanics; cell mechanical communication; directional migration; Jurkat T cells, mechanotaxis

1. Introduction

Cell mechanical communication has emerged as a key research frontier, encompassing cell-cell mechanical interactions, cell-extracellular matrix (ECM) mechanical crosstalk, and mechanosensitive channel signaling. These processes control fundamental immune functions and disease progressions. In recent decade, a variety of molecular and signaling pathways related to mechanical communication have been elucidated. At the immune synapse, T cells bind to ICAM-1 of target cells through LFA-1, transmitting mechanical signals to enhance immune responses[1]. When ECM stiffness is increased, mechanical tension inhibits the Hippo pathway, promoting YAP/TAZ nuclear translocation and fibrotic or tumorigenic gene expression[2]. The mechanosensitive ion channel Piezo1 converts mechanical forces into biochemical signals to drive tumor angiogenesis [3]. Many mechanical signaling pathways affect the mechanical communication of cells.

Chemokines are important regulatory factors in immune response. In most theories, the directional aggregation of immune cells depends on chemical factors, namely chemokines. CCL19 and CCL21 are involved in immune surveillance by directing T cell migration to lymph nodes through the receptor CCR7[4]. On the surface of endothelial cells at sites of inflammation, LFA-1 mediates T cell adhesion and crawling on blood vessels for transendothelial migration[5]. CX3CR1, a receptor for the chemokine CX3CL1, plays an important role in the regulation of inflammatory responses, including monocyte homeostasis and macrophage phenotype and function[6].

Mounting evidence indicates that immune cells are also regulated by mechanical cues. Fluid shear stress enhances NK cell cytotoxicity against circulating tumor cells through NKG2D-dependent mechanosensing [7]. $CD8^+$ T cells sense substrate stiffness via Piezo1, and excessive stiffness induces $CD8^+$ T-cell exhaustion [8]. Memory B cells exhibit high sensitivity to mechanical forces, whereas naive B cells display a threshold-dependent mechanosensory response relying on Myosin IIA and integrins [9]. These findings highlight the dual control of immune cells by chemical and mechanical signals.

Despite advances in cancer immunotherapy, the mechanistic basis underlying force-modulated immune responses remains poorly defined. Piezo1 deficiency alleviates lung inflammation in bacterial infection models[10]. Mechanically sensitive immune cells are activated to different degrees by different mechanical signals[11]. Some immune cells are sensitive to mechanical stimuli and matrix stiffness and thus affect the immune response[12]. Mechanical cancer therapies such as focused ultrasound (FUS) trigger immune activation through thermal and mechanical effects, enhancing vascular permeability, T-cell infiltration, and tumor suppression[13]. Engineered ultrasound-responsive T cells further highlight the translational potential of mechanoimmunotherapy[14]. With the development of various researches, mechanoimmunotherapy will become more and more important.

Macrophages play a central role in immune responses by secreting pro-inflammatory cytokines (e.g., IL-6, TNF-α) to recruit immune cells to inflammatory foci[15]. Integrin-ECM adhesion signals initiate signaling pathways that affect macrophage activation[16]. Increased matrix stiffness leads to upregulation of Piezo1 expression and activation of the mechanotransduction effector YAP, which regulates macrophage polarization[17]. Macrophages can track cancer cells via collagen-transmitted mechanical signals, likely because cancer cells generate stronger mechanical cues via enhanced motility and ECM remodeling[18]. However, whether mechanical signals direct the migration of suspension immune cells remains unclear.

In this study, we applied a collagen hydrogel-based model to dissect the effects of mechanical cues (using airway smooth muscle cells and lung cancer cells as force sources) on the directional migration of suspension immune cells, using Jurkat T cells as a paradigm and macrophages RAW264.7 as a positive control. We show that mechanical cues,

in addition to the traditional chemotactic factors that drive cell migration, are also effective in guiding T cell directional migration. These findings revise the classical chemokine-centric model of immune cell migration and provide a theoretical foundation for integrating mechanical immune regulation with chemotherapy.

2. Materials and Methods

2.1. Cell culture and regents

Human airway smooth muscle cells (hASMC), Jurkat T cells, human lung cancer cells A549, and murine macrophages RAW264.7 were purchased from BeiNa Biology. All types of cells were cultured in mediums supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. hASMC cells were maintained in low glucose-type DMEM medium, Jurkat T cells in RIPM 1640 medium, A549 in F12 medium, and RAW264.7 in high glucose DMEM medium. Cells were cultured in a humidified cell incubator containing 5% CO_2 at 37°C. Jurkat T was cultured on a mini-shaker (~20 rpm) to maintain suspension growth within the incubator.

All cell culture mediums, FBS, Lipofectamine 3000, and Opti-MEM were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Rat type-I collagen was from Advanced Biomatrix and Matrigel™ from BD Biotechnology (CA, USA). Cytochalasin D (Cyto D) was from Sigma-Aldrich.

2.2. Constructing hydrogel model for two-type cell-cell mechanical interactions

To describe the experimental scheme, hAMSC was used as the force-generating cells and Jurkat T was used as the experimental immune cells. hAMSC cells had larger size than Jurkat T, and to further distinguish them under the microscope imaging, Jurkat T was stained with live cell dye CellTracker Red. All procedures were performed on ice. f 5 mL. To make polydimethylsiloxane (PDMS), the two liquid components from the Sylgard 184 kit (Dow Corning) were well mixed at a 10:1 mass ratio in a petri dish followed by curing at 70°C. A PDMS mold (inner diameter ≈1 cm, outer diameter ≈1.5 cm, thickness ≈2 mm) was attached onto the glass bottom of a confocal dish. Rat-tail type I collagen (3.9 mg/ml) was mixed with neutralizing buffer in a 9:1 ratio, and diluted to 1 mg/mL with PBS solution. The prepared collagen solution was added into the PDMS ring by using a 10 μl pipette, and spread several times until the inner plane of the ring was covered evenly. Then, the hydrogel model was incubated at 37°C for 15 min to allow gelation.

Jurkat T cells were stained with CellTracker Red: 3 mL of the cells from suspension culture were centrifuged, resuspended with 2 ml of serum-free RIPM 1640 medium containing 5 μl of CellTracker Red, and then incubated in the cell culture incubator for 30 min. After centrifugate, cells were adjusted to 5×10^6/ml in complete RIPM 1640 medium. hASMC cells were trypsinized, and diluted to 1×10^5 cells/mL. 20 μl hASMC cell suspension was seeded onto the collagen gel evenly within the PDMS ring, and then the dish was placed within a six-well holder (Karl Zeiss) and incubated for 1 h to allow cell attachment. Then, 20 μl of stained Jurkat T suspension was added and incubated for another 1 h. a 1:1 mixture of RIPM 1640 medium and low-sugar DMEM medium (10% FBS) was made for hASMC and Jurkat T co-culture in experiments. 2 mL of the mixture medium were added gently along the dish edge without disturbing the cells, and samples were subjected to time-lapse imaging in the live cell microscope workstation.

Four cell interaction pairs were tested: Jurkat T + hASMC, Jurakt T + A549, RAW264.7 + hASMC, RAW264.7 + A549, by following the similar preparations. As inert negative controls, magnetic beads (~5 μm in diameter) were added at similar density to replace the immune cells. For 3D collagen embedding experiments, immune cells were mixed with

collagen solution (1 mg/mL) and overlaid on collagen gels pre-seeded with force-generating cells (hASMC or A549), followed by gelation for 15 min and medium addition.

2.3. Glutaraldehyde crosslinking of the hydrogel

Glutaraldehyde crosslinking of the hydrogel can reduce traction force transmission through the matrices, which served as a negative condition in this work. Briefly, the 1.0 mg/mL collagen hydrogel was treated with 0.05% glutaraldehyde solution for 15 minutes and then neutralized with Tris-HCl solution for 1 hour, followed by three times of PBS buffer washings (5 min each time). The method hereafter is the same for two-type cell seedings.

Alternatively, to mimic in vivo basement membrane matrices, the experimental condition was examined on the hydrogel of 50% Matrigel (~5 mg/mL) supplemented with 1.0 mg/mL type I collagen (COL). To compare with the untreated normal condition, the"Matrigel + COL" hydrogel was crosslinked with 0.05% glutaraldehyde solution, or the force-generating cells hASMCs were pre-treated with Cyto D (1.0 μg/mL) for 1 h to reduce cell contraction force. The co-culture of hASMC and Jurkat cells were also seeded on glass surface coated with 1.0 % "Matrigel + COL" diluted solution, as non-traction force transmission control.

2.4. Live cell microscope imaging

Time-lapse imaging was carried out on a Zeiss observer.Z1 live-cell workstation equipped with a temperature- and CO_2-controlled chamber (37°C, 5% CO_2). The cell sample dishes were placed into a six-well holder within the chamber. Images were acquired at 1 h intervals for 36 h. Stained Jurkat T cells were visualized using a Rhodamine channel.

2.5. Cell Trajectory Tracking and Analysis

Time-series images were imported into ImageJ. Scale calibration was performed via Analyze > Set Scale by clicking the Straight Line, and then Set Scale in the Analyze column to convert visual distance to real size. Cell migration was tracked using Plugins > Manual Tracking to generate coordinate data by using Add track frame by frame to obtain cell trajectory results. The digital data files were imported into MATLAB 2015b software to determine the move rate, velocity, trajectory, and travel distance as described previously (reference).

2.6 Statistical Analysis

Data were analyzed and plotted using GraphPad Prism, and Origin2020. Values are presented as mean ± standard deviation (S.D.). Student's t-test was used for two-group comparisons; one-way ANOVA for multiple groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ were considered statistically significant.

3. Results

3.1. Jurkat T Cells Exhibit Directional Migration Toward Force-Generating Cells on 2D Collagen

In the traditional view, the directional migration of immune cells is mainly affected by chemical factors, and there are few studies on the influence of mechanical factors, especially for the study of suspended immune cells to be promoted. To investigate whether the directional migration of suspension-like immune cells would be affected by mechanics, we constructed an experimental model with hASMC and A549 as force source cells capable of generating force signals on 1.0 mg/mL collagen hydrogel, and used micro-bead as

inert control and error comparison of collagen deformation induced by cell strains. One study found that macrophages were able to track cancer cells through force signals from collagen matrix[18], so RAW264.7 was used as a positive control, and Jurkat T was used as the experimental group to explore whether it is subject to mechanical regulation by the force-generating cell attraction.

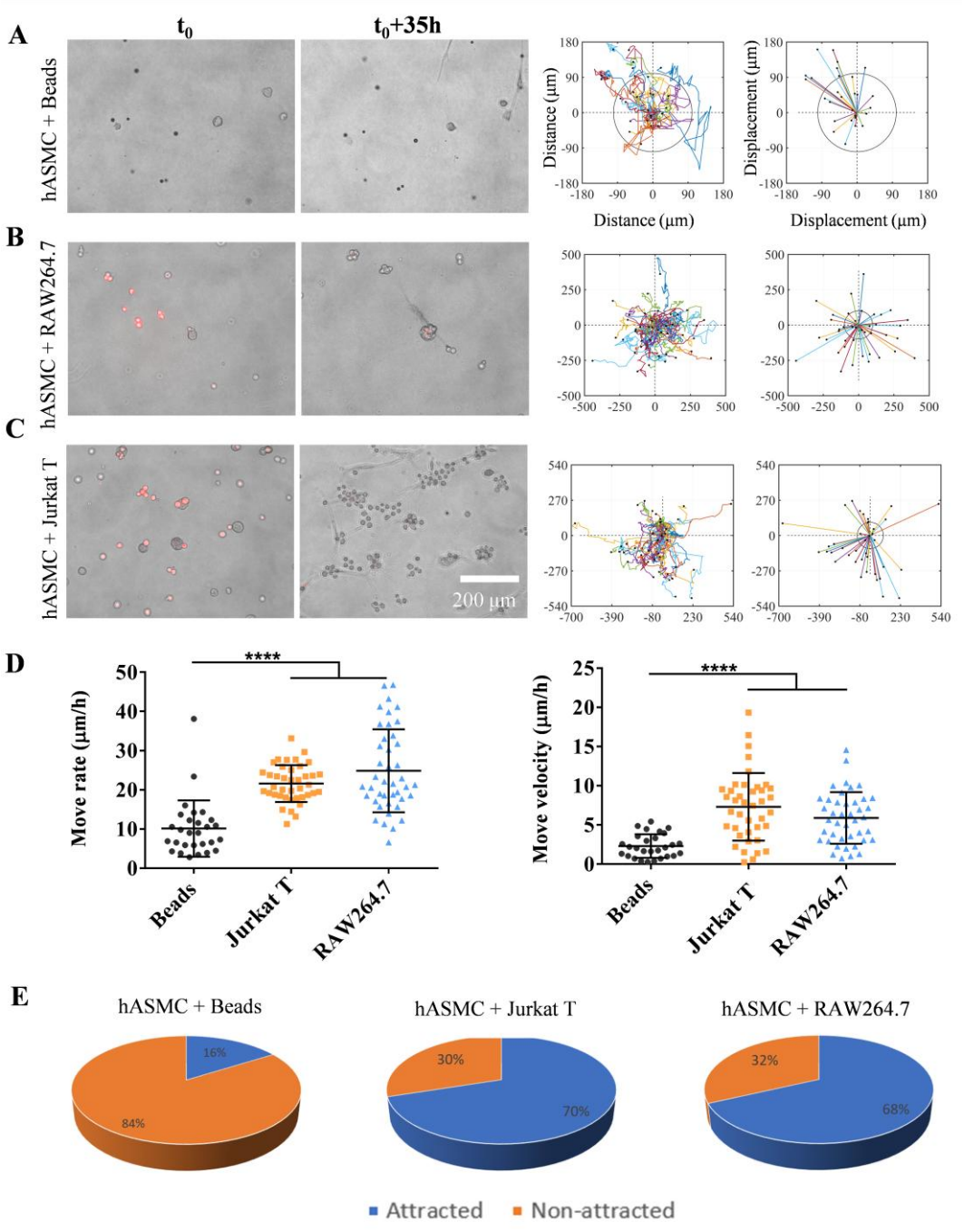

Figure 1. Directional migration of Jurkat T cells toward force-generating hASMCs on 2D collagen hydrogel. (A-C) Representative cell images and migration trajectories of Jurkat T cells, RAW264.7 macrophages, and inert magnetic beads in co-culture with hASMCs, respectively. (D) The migration rate and velocity of different experimental groups, and their statistical comparisons of. (E) The probability of migration of different experimental subjects to the force-generating cells hASMC.

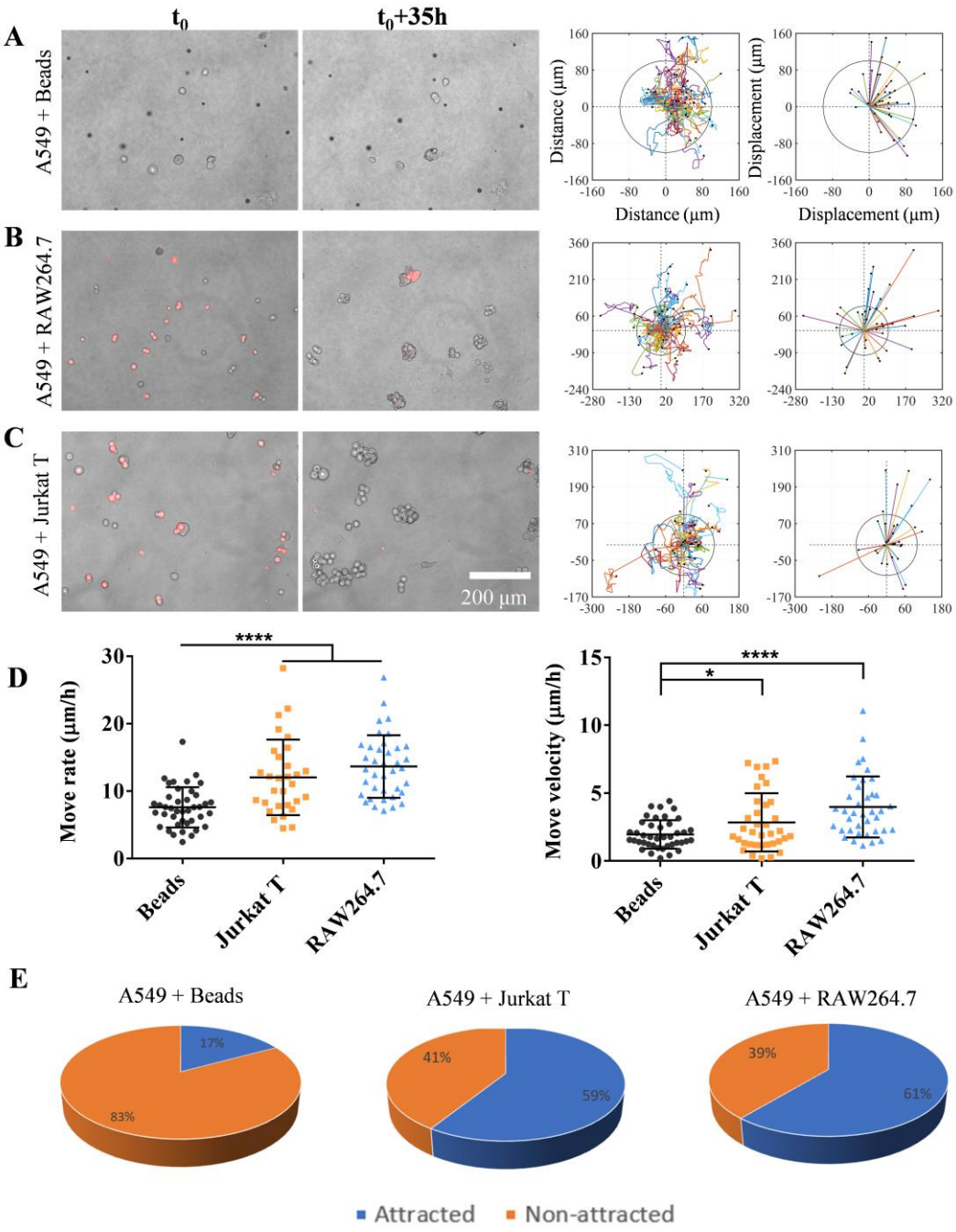

Figure 2. Directional migration of Jurkat T cells toward force-generating A549 cells on 2D collagen hydrogel. (A-C) Representative cell images and migration trajectories of Jurkat T cells, RAW264.7 macrophages, and inert magnetic beads in co-culture with A549 cells, respectively. (D) The migration rate and migration velocity of different experimental groups, and their statistical comparisons of. (E) The probability of migration of different experimental subjects to the force-generating A549 cells.

As shown in Figures 1A-C & 2A-C, we co-cultured Jurkat T cells (stained with CellTracker Red) with force-generating hASMCs (Figure 1) or A549 cells (Figure 2). RAW264.7 macrophages (stained with CellTracker Red) served as a positive control, and inert magnetic beads as a negative control. Migration was quantified within a 300-μm region centered on the force-generating cells. The results of quantitative data showed that Jurkat T could migrate to the force-generating cells on the 2D collagen hydrogels, similarly to RAW264.2 macrophages (Figures 1D & 2D). By counting the directional migrations of microbeads, RAW264.7 and Jurkat T within a circle of 300 μm diameter centered on the

force source cell, over 60% of Jurkat T cells and RAW264.7 macrophages migrated directionally toward force-generating cells, whereas fewer than 20% of beads showed directed movement (Figures 1E and 2E). These results confirm that suspension immune cells can sense and migrate toward the force-generating cells. Hence, it is speculated that the suspension-like immune cells are regulated by chemical signals and mechanical signals in the process of participating in the immune response, which makes it more accurate to track abnormal cells.

3.2. 3D Collagen Matrices Attenuate Jurkat T-Cell Directional Migration

In order to simulate different microenvironments, the cells were encapsulated within 1.0 mg/mL collagen hydrogel for experimental observation. The procedure was similar to that described above, except that the stained immune cells were mixed with the non-coagulated collagen and plated onto 1.0 mg/mL collagen seeded with hASMC or A549 on the surface, and after 15 min solidification at 37 °C, the medium was added.

As shown in Figures 3 and 4, directional migration of Jurkat T cells toward force-generating cells was significantly reduced in 3D, accompanied by lower migration probability, speed, and displacement, which may be due to the existence of type I collagen in the migration process. In comparison to Jurkat T, RAW264.7 macrophages showed higher migration efficiency and probability toward force-generating cells (Figures 3D&E and 4D&E). The impairment may result from the physical barrier imposed by dense collagen fibers, which are known to restrict T-cell infiltration into tumors [19].

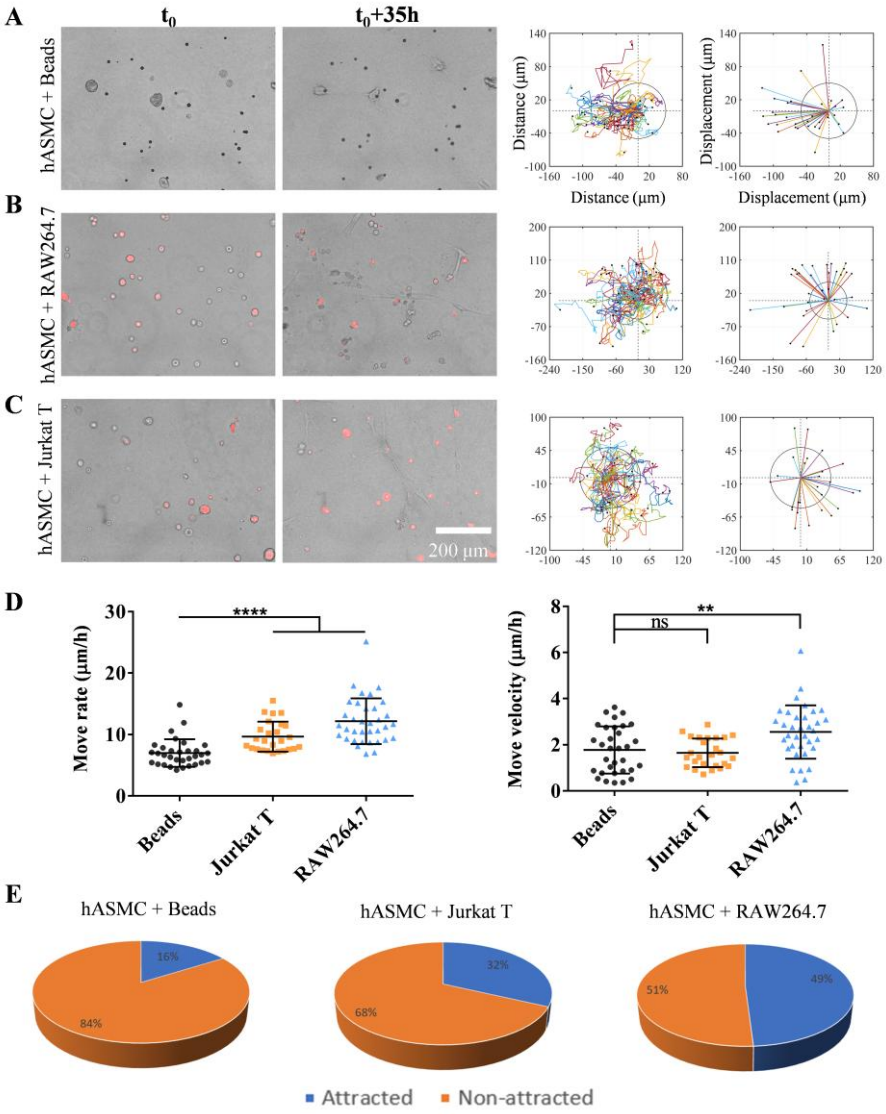

Figure 3. Impaired migration of Jurkat T toward hASMC in the interior of 1 mg/mL collagen hydrogel. Quantitative analysis of migration probability, speed, and displacement of Jurkat T cells in 3D collagen matrices. hASMC was used as the force source cells, and the cells were encapsulated in collagen. (A-C) Experimental results and cell trajectory ensemble plots with Beads, RAW264.7, Jurkat T, respectively. (D) The migration rate and speed of different experimental groups, and (E) the probability of different experimental subjects migrating to the force-generating hASMCs.

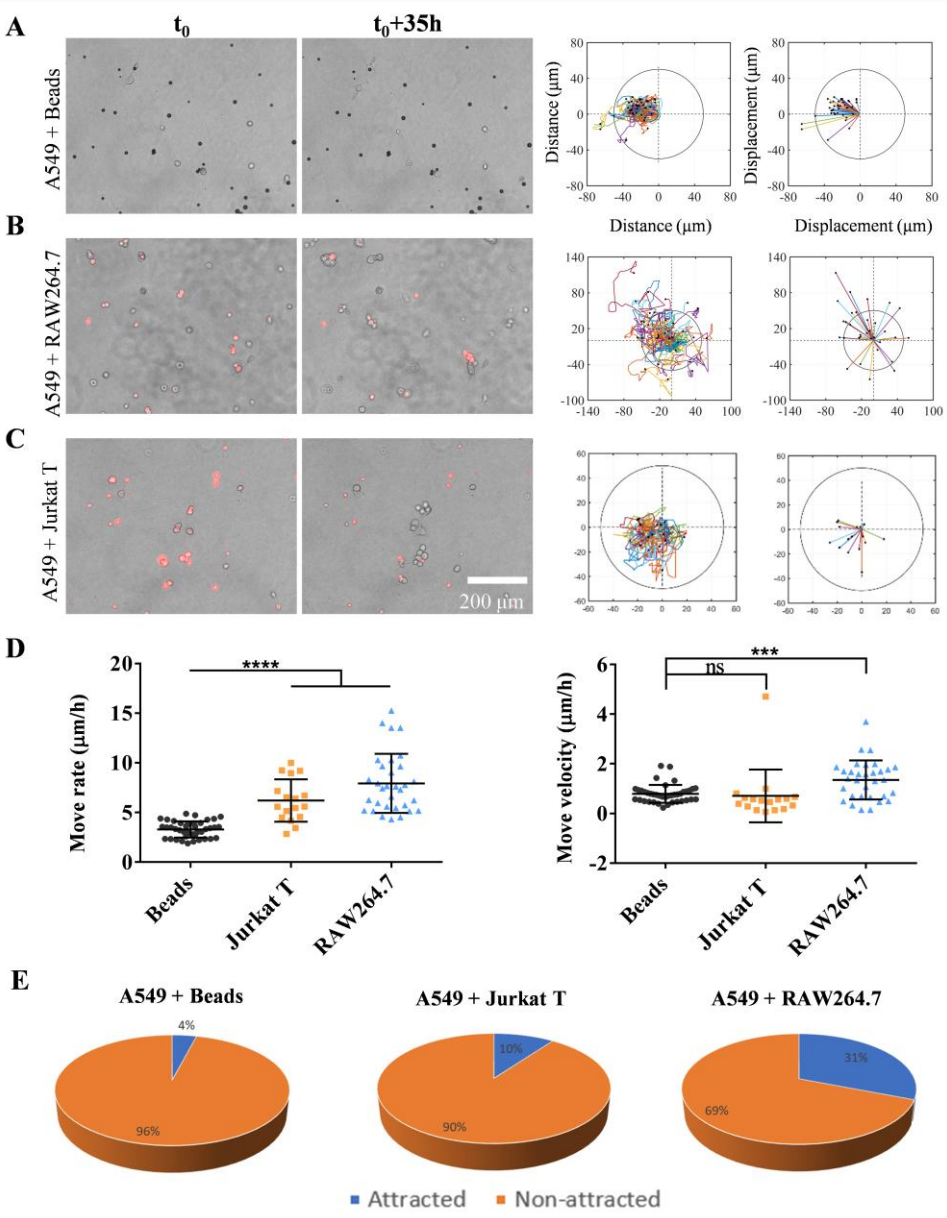

Figure 4. Representative migration trajectories of Jurkat T cells toward A549 cells in 3D collagen matrices. Using A549 cells as the force source, the cells were encapsulated in 1 mg/mL collagen. (A-C) Experimental results and cell trajectory ensemble plots with Bead, RAW264.7, Jurkat T, respectively. (D) The migration rate and migration velocity of different experimental groups. (E) The probability of migration of different experimental subjects to the force source A549 cells.

3.3. Weakened Directional Migration of Jurkat T toward Force-Generating Cells on Glutaraldehyde-Crosslinked Hydrogel

In order to verify whether there are mechanical factors involved in the migration process of T cells, the collagen surface was cross-linked and solidified to inhibit traction

force transmission. The 1 mg/mL collagen hydrogel was treated with 0.05% glutaraldehyde solution ("$OHC(CH_2)_3CHO$") for 15 minutes and then neutralized with Tris-HCl solution to restore the neutral collagen pH value, the method hereafter is the same as before. As shown in Figure 5(A-C), the experimental results showed that the probability of directional migration to force-generating cells was significantly reduced. These phenomena indicate that the directional migration of T cells is also affected by reducing the influence of mechanical cues, which may be one of the important factors in the directional migration of T cells. As examined in our previous work, glutaraldehyde crosslinking caused the hydrogel stiffer [20], which could lead to enhanced random mobility of the cells.

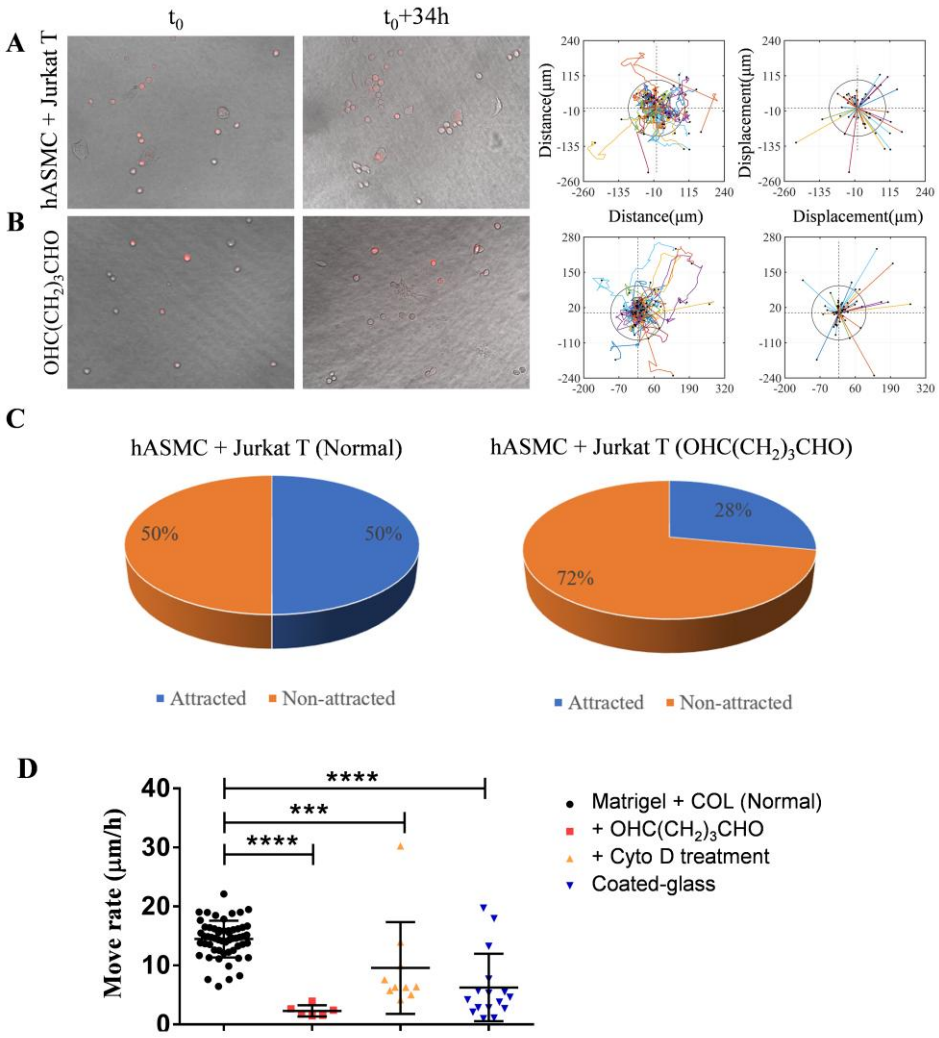

Figure 5. The effect of glutaraldehyde-mediated hydrogel crosslinking on Jurkat T-cell mechanotaxis. Experimental model of hydrogel surface after curing using glutaraldehyde cross-linking, and quantitative analysis and representative trajectories showing different migration after hydrogel crosslinking. (A, B) Ensemble plot of experimental results and cell trajectories for untreated control group and experimental group treated with glutaraldehyde ($OHC(CH_2)_3CHO$). (C) Probability of migration of Jurkat T toward force-generating hASMC cells in the control and experimental groups. (D) The migration rates of Jurkat T toward force-generating hASMC on 2D "Matrigel + COL" hydrogel in the normal and experimental groups (glutaraldehyde crosslinking, hASMC with Cyto D treatment, or on the glass surface coated with 1.0% of "Matrigel + COL" solution).

In vivo environment, T cells often migrate through or crawl on basement membrane, one layer of matrices underneath epithelial tissues [21-23]. We also tested the mechanical

role in Jurkat T migration on the hydrogel matrices containing 50% Matrigel (~5 mg/mL, mimicking basement membrane components) and 1.0 mg/mL type I collagen (COL), as described in our previous work [24]. To validate the role of matrix mechanics, we stiffened the "Matrigel + COL" using glutaraldehyde crosslinking, or inhibited hASMC contraction with Cyto D treatment. As a negative control without traction force transmission through matrices, hASMC and Jurkat T were co-cultured on the glass surface coated with 1.0% of "Matrigel + COL" solution (~60 μg/mL). The results showed that in comparison to the normal condition, the migration of Jurkat T cells was nearly abolished either after glutaraldehyde crosslinking of the hydrogel, or hASMC treatment with Cyto D, or on glass-surface (Figure 5D). This result further demonstrates that dynamic matrix mechanics are essential for T-cell mechanotaxis in this experimental model.

3.4. T -Cell Migration Is Modulated by the Type of Force-Generating Cells

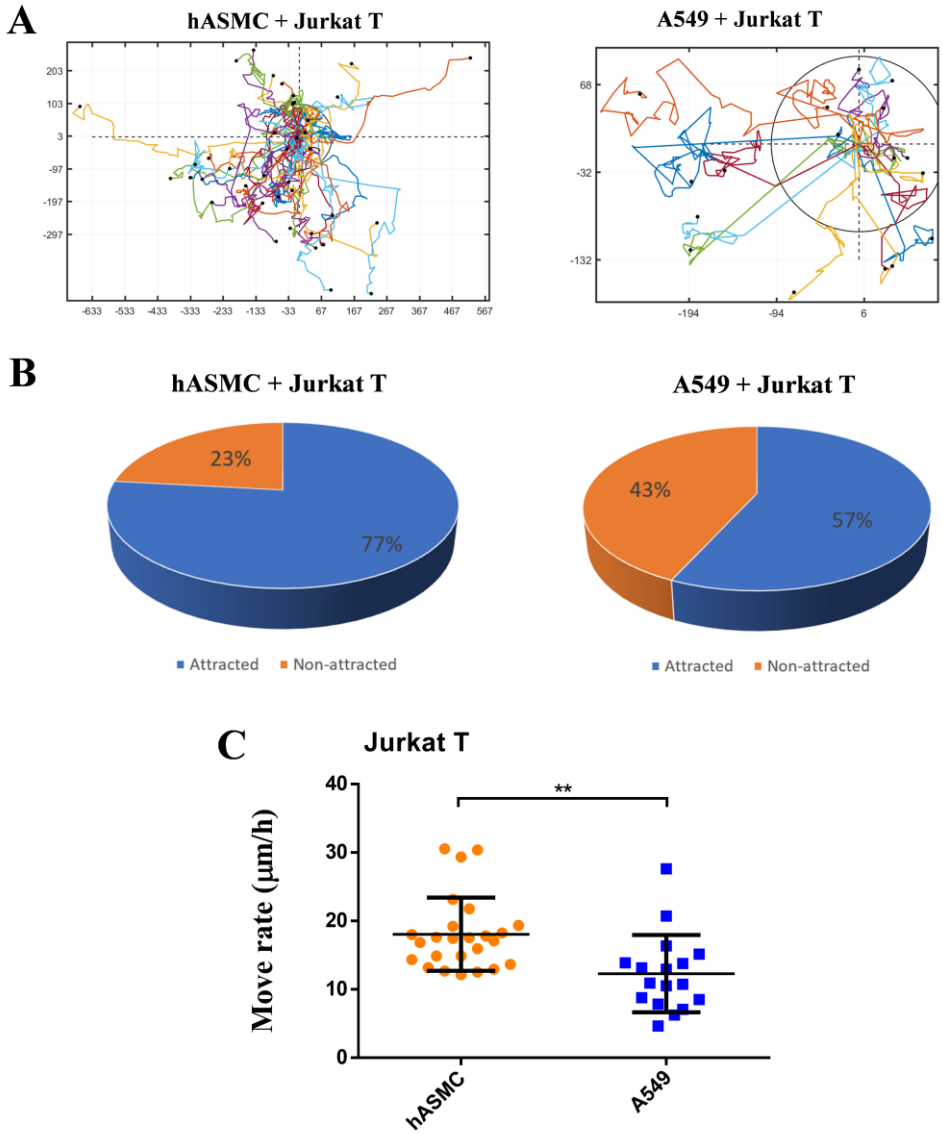

Figure 6. The modulation of Jurkat T migration by different types of force-generating cells. Jurkat T and hASMC or A549 were co-cultured on 2D collagen hydrogel, followed by time-lapse imaging and migration quantification. Beads and RAW264.7 groups served as inert and positive controls. (A, B) Quantified trajectories and migration rates of Jurkat T cells paired with hASMC or A549. (C) Attraction probability of Jurkat T migration toward force-generating hASMC or A549.

Different microenvironments may also affect the migration ability of Jurkat T. In the two-pair experimental groups using hASMC or A549 cells as force-generating cells (Figures 1 & 2), Jurkat T showed relatively higher migration efficiency and larger attraction probability in hASMC groups than in A549 groups, which difference in paired comparisons was demonstrated in Figure 6A-C. It is possible that different microenvironments also have some effect on the ability of cells to plan their routes. As reported from previous work this functional difference may stem from the difference in the mechanism of mechanical signal generation between the two cell types, with airway smooth muscle tending to produce sustained and stable homeostatic contractions, whereas cancer cells may exhibit dynamic contractile features [25]. The way and magnitude of the force produced by the two types of cells is slightly different, likely attributing Jurkat T's migration ability in mechanotaxis. Hypothetically, the observed difference in Jurkat T migrations likely arise from cell-specific mechanical signaling patterns and ECM remodeling activities.

4. Discussion

Our study demonstrates that Jurkat T cells undergo robust directional migration toward force-generating cells on type I collagen hydrogel, similar to macrophages, a response not observed in inert beads. Glutaraldehyde-mediated collagen crosslinking eliminated this mechanotaxis, confirming that dynamic matrix mechanics are required for T-cell mechanosensing. These findings add new insights into the conventional view that immune cell migration is controlled by chemokine gradients.

Cell-cell mechanical communications can mechanically attract direction migration of adhesive macrophages [26-27], while T cells under suspension growth in circulation system were not characterized for this type of mechanical attraction. Our experiments confirmed that T cells on the surface of type I collagen matrix showed a remarkable characteristic migration with their trajectories clearly pointing to the mechanically active target cells. In contrast to the negative results in the inert micro-beads group, this phenomenon confirms that T cell migration is mediated by cell-cell mechanical interactions. Further, the network of collagen fibers was solidified by glutaraldehyde crosslinking to inhibit long-range traction force transmission, and it was found that the directional migration of T cells was significantly decreased, which directly revealed that the mechanical properties of collagen fibers with traction force transmission is the key material basis for T cells to sense the mechanical signals of the microenvironment, and reducing the influence of mechanical factors will reduce the directional mobility. These physical properties may precisely regulate the migration behavior of immune cells through specific mechanotransduction pathways.

The divergent responses of Jurkat T migration to hASMCs and A549 cells reflect distinct mechanical microenvironments: hASMCs produce steady contractile forces, whereas A549 cells generate dynamic forces accompanied by MMP-2/9-dependent ECM degradation [28-30]. These distinct mechanical signals may activate different downstream pathways, which may provide important clues for understanding the tissue microenvironment-specific mechanotransduction mechanisms, and new insights into the mechanism of tissue-specific mechanotransduction. It is suggested that the dynamic characteristics of mechanical signals may be the key factors affecting the regulation mode of cell migration behavior under different physiological or pathological conditions. The ability of tumor cells and smooth muscle cells to remodel the extracellular matrix is also very different. Cancer cells usually express matrix metalloproteinase such as MMP-2 and MMP-9 significantly, which can degrade collagen fibers efficiently, thus constructing a matrix stiffness microenvironment with gradient characteristics, while smooth muscle cells do not have

this ability of cancer cells [31]. This distinct remodeling mechanism may also influence the topological characteristics of T cell migration pathways.

This study elucidates the universal mechanism of mechanical force perception in the process of immune cell chemotaxis, breaking through the theoretical framework of chemotactic factors dominating immune cell migration in traditional theories. This study not only reasonably explains the limitations of chemotactic factors in predicting the trajectory of immune cells in previous studies, but also provides a new perspective for understanding the migration mechanism of immune cells.

This study innovatively reveals the causal relationship between mechanical microenvironment heterogeneity and T cell migration behavior, and proposes a theoretical hypothesis that mechanical force heterogeneity can drive T cells to adopt differentiated migration strategies. The theory has the potential to provide innovative explanations for key clinical issues, and mechanical signals can also affect immune cell migration, which can guide immune cells to aggregate more precisely. However, in the dense microenvironment, it may also lead to the dysfunction of T cell migration and then cause immune escape. This study is expected to fill the important gap in the field of immunomechanics.

5. Conclusions

The study used live-cell imaging to Quantitative analysis that mechanical cues, in addition to traditional chemoattractant gradients, are also effective at directing T-cell migration at the single-cell scale. T-cell mechanotaxis is further modulated by force-generating cell type and matrix dimensionality. The study found that despite the heterogeneity of T cells in response to mechanical stimuli from different sources, such as lung cancer cells and airway smooth muscle cells, this finding revises the classical chemokine-dominant model of immune cell migration and explain the impaired immune infiltration in stiff fibrotic and tumor stroma.

Author Contributions

Conceptualization, M.O. and L.D.; methodology, Q.Z., J.G. and M.O.; validation, Q.Z., H.L., J.G. and L.C.; formal analysis, Q.Z. and H.L.; investigation, Q.Z.; resources, M.O., L.D., J.G. and B.L.; data curation, Q.Z., H.L. and M.O.; writing—original draft, H.L. and Q.Z.; writing—review & editing, M.O. and L.D.; visualization, Q.Z. and H.L.; supervision, M.O.; project administration, M.O. and L.D.; funding acquisition, M.O. and L.D. All authors have read and approved the final manuscript.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

All data are available from the authors upon reasonable requests.

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Conflicts of Interest

The authors declare no conflict of interest.

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