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Microfluidic Single Cell Arrays to Interrogate Signalling Dynamics of Individual, Patient-derived Hematopoietic Stem Cells

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Abstract

Stem cells hold great promise as a means of treating otherwise incurable, degenerative diseases due to their ability both to self-renew and differentiate. However, stem cell damage can also play a role in the disease with the formation of solid tumors and leukemias such as chronic myeloid leukemia (CML), a hematopoietic stem cell (HSC) disorder. Despite recent medical advances, CML remains incurable by drug therapy. Understanding the mechanisms which govern chemoresistance of individual stem cell leukemias may therefore require analysis at the single cell level. This task is not trivial using current technologies given that isolating HSCs is difficult, expensive, and inefficient due to low cell yield from patients. In addition, hematopoietic cells are largely non-adherent and thus difficult to study over time using conventional cell culture techniques. Hence the need for new microfluidic platforms that allow the functional interrogation of hundreds of non-adherent single cells in parallel. We demonstrate the ability to perform assays, normally performed on the macroscopic scale, within the microfluidic platform using minimal reagents and low numbers of primary cells. We investigated normal and CML stem cell responses to the tyrosine kinase inhibitor, dasatinib, a drug approved for the treatment of CML. Results showed that the primary cells tolerated the microfluidic environment well and continued to proliferate as normal. Dynamic, on-chip three-color cell viability assays revealed that differences in the responses of normal and CML stem/progenitor cells to dasatinib were observed even in the early phases of exposure, during which time normal cells exhibit a significantly elevated cell death rate, as compared to both controls and CML cells. Further studies show that dasatinib does, however, markedly reduces CML stem/progenitor cell migration *in situ*.

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† Electronic Supplementary Information (ESI) available: Four movies illustrating cell loading of device, cell division, apoptosis, and cell

migration are included in ESI materials. Please see individual captions for details. See DOI: 10.1039/b000000x/

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Introduction

Possessing the unique ability to both self-renew as well as differentiate into multiple cell phenotypes, stem cells hold enormous potential to one day provide a means of treatment for otherwise incurable diseases such as neurological disorders, diabetes, and cancer. Thus, great importance is laid upon understating the cellular mechanisms which mediate stem cell function as well as their fate. In addition to enhancing knowledge of cell biology system dynamics, this would also allow the development of novel stem cell based therapeutics for degenerative disorders and malignancies.

Chronic myeloid leukemia (CML), a hematopoietic stem cell (HSC) disorder, results from a reciprocal translocation between chromosome 9 and 22 [t(9;22)] leading to the formation of the BCR-ABL oncoprotein.^{1,2} HSCs are somatic cells that can differentiate into any hematopoietic cell type depending upon the conditions in the stem cell niche (i.e. the bone marrow microenvironment). Despite impressive rates of remission with BCR-ABL targeted tyrosine kinase inhibitors (TKI) imatinib

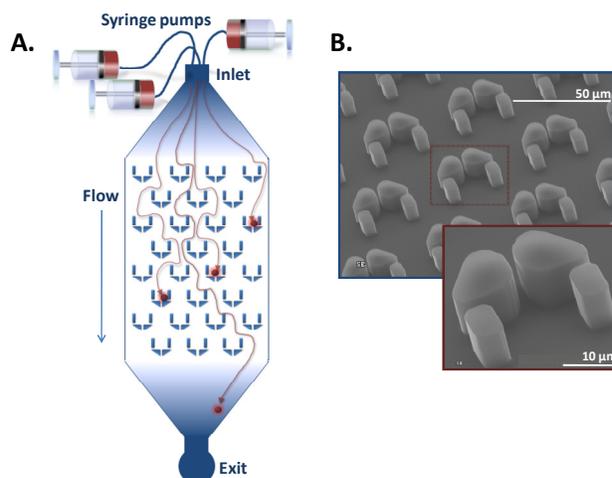


Figure 1: Device illustrations. (A) Schematic representation of microfluidic device concept, including illustration of cell trapping by hydrodynamic flow. Actual device contain upwards of 440, 18 μm x 18 μm x 10 μm cell traps. (B) Scanning electron microscope images of PDMS cell traps.

(Gleevec), dasatinib (Sprycel), and nilotinib (Tasigna), molecularly detectable disease remains present in the majority of patients. It is hypothesized that this molecular disease persistence results from a population of quiescent CML stem cells which are resistant to therapy.³ The signaling pathways which enable CML stem cell survival in the presence of TKIs are still unknown, but are of great interest in terms of identifying curative treatment strategies for CML as well as understanding basic stem cell biology. It is necessary, in such investigations, to examine signalling dynamics on the single cell level in order to identify variations in cellular characteristics which protect certain cells, but not others, against drug-induced cell death.

Because hematopoietic cells are non-adherent, it is difficult to monitor individual cells over time using conventional, macroscopic cell culture techniques. Furthermore, normal and leukemic HSCs occur in small numbers within the body and are normally located deep within the bone marrow rather than in circulation. Thus, isolating these stem cells is not trivial and obtaining quantities sufficient for bench-top scale experiments remains a significant challenge in studying drug resistant cells. Many of these challenges, however, may be easily overcome through the application of microfluidic, lab-on-a-chip technology.

Microfluidic based cell culture platforms are inherently well-suited for working with populations of rare cell fractions. The microfluidic platform used in this investigation is based upon a platform described previously,^{4, 5} and is illustrated in Figure 1. This platform is specifically designed to accommodate hundreds of individual, non-adherent cells in parallel using a passive trapping mechanism that functions not unlike capturing balls in a pachinko game (See ESI Movie 1 for video of cells loading within the device). As opposed to bench-top protocols which call for millions of cells per assay, each experiment taking place within the microdevice, with a total volume of approximately 20 nl, requiring of the order hundreds of cells and only minimal amounts of reagents. Given that only a few hundred thousand CD34+ hematopoietic stem cells might be collected from a single patient, performing experiments using the microfluidic platform not only provides the advantage of single cell resolution, but also the ability to run several assays on the same batch of patient-derived cells. In addition, the ability to sensitively control the fluidic environment on-chip has the potential to engage multiple assays simultaneously within a single device. These attributes make the application of microfluidic technology to study stem cell signalling events an attractive means of gaining new insight into single cell dynamics.

Generally, microfluidic analysis of stem cells utilized cells derived from murine sources or immortalized human stem cell lines.⁶⁻⁹ Because immortalized cell lines possess mutations to allow continuous growth *in vitro*, their likeness to *in vivo* response to environmental influence will differ from primary cells and thus be somewhat less useful to physiologic extrapolation. In addition, data from murine cells is notoriously difficult to interpret in the human context. For these reasons, we have applied our microfluidic platform to study single, patient-derived CD34+ stem/progenitor cells.

The following experiments demonstrate the ability to perform assays normally carried out on the macroscopic level within our microfluidic platform. We investigate patient-derived normal and CML stem cell responses to the TKI dasatinib, a small molecule inhibitor approved for treatment of imatinib resistant CML. Interestingly, not only are the assays successful in achieving the

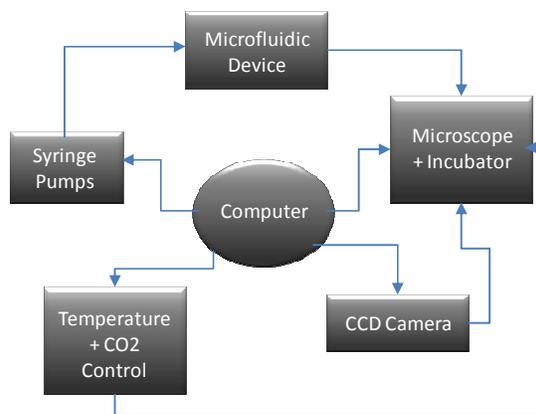


Figure 2: Experimental setup. Flow chart illustrating hardware connectivity in typical experimental setup.

predicted results, but the ability to monitor single cells over time also provides additional insight into cellular behaviors which are not apparent when using conventional techniques.

Experimental

Cell Culture

Fresh leukapheresis or peripheral blood samples were obtained with written informed consent and approval of the Local Ethics Committees from patients with newly diagnosed CML and normal donors of peripheral blood stem cells. Samples were enriched for CD34+ cells using CliniMACS (Miltenyi Biotech Ltd) according to the manufacturer's instructions and cryopreserved in 10% (v/v) DMSO in ALBA (4% [w/v] Human Albumin Solution. Scottish National Blood Transfusion Service).

Upon thawing, CD34+ cells were cultured in serum free medium consisting of Iscove's Modified Dulbeccos Medium (IMDM, Invitrogen) supplemented with serum substitute (bovine serum albumin (BSA), insulin, transferrin (BIT, Stem Cell Technologies), L-glutamine (Invitrogen), penicillin/streptomycin (Invitrogen), 0.1 μM 2-mercaptoethanol (Sigma-Aldrich). In addition, media contained a high concentration five growth factor cocktail comprised of 20 ng ml^{-1} IL-3, 20 ng ml^{-1} IL-6, 100 ng ml^{-1} stem cell factor, 100 ng ml^{-1} Flt-3 ligand, and 20 ng ml^{-1} granulocyte-colony stimulating factor, all from Stem Cell Technologies. Cells were maintained in 37°C incubator with 5% CO_2 .

Microfluidic Preparation

Multianalyte nanophysiometer SU-8 masters were generated using soft lithographic techniques as previously detailed.^{4, 5} Microfluidic devices were cast in polydimethylsiloxane (PDMS, Dow Corning) and irreversibly bonded to glass coverslips using surface treatment of plasma oxidation. Devices were sterilized/cleaned immediately prior to use by immersion for 10 minutes in sonic bath containing methanol. Sterile cell media was then used to flush the device and ensure that no air or methanol remained. Gas-tight syringes (Hamilton) were fitted to 50 μm inner diameter PEEK (poly-ether-ether-ketone) tubing (VICI Precision Sampling, Inc.) and controlled using microsyringe pumps (Harvard Apparatus). Cells were transferred to the device accordingly: cells were aspirated directly from centrifugation pellet into the PEEK tubing (50 μm ID, 360 μm OD) which was

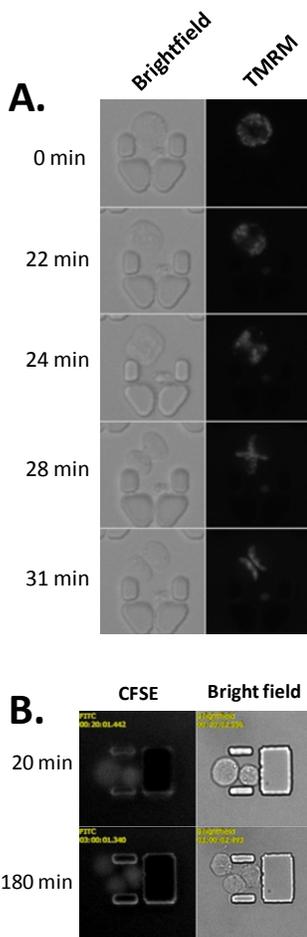


Figure 3: Monitoring stem cell division. (A) Normal CD34⁺ HSCs stained with TMRM to label mitochondria. During cell division, mitochondria proceed from being well dispersed within the cell to polarizing at the cleavage furrow during mitosis in such a manner that upon pinching of cell membrane, approximately equal numbers of mitochondria are split among daughter cells. (B) CML CD34⁺ hematopoietic stem cells stained with CFSE to monitor cell division, again with each daughter cell retaining approximately half of the fluorescence.

subsequently inserted into the device inlet port. Fluid flow rates of 50 nl min⁻¹ were sufficient to load, trap, and perfuse cells for the duration of the experiment (unless mentioned otherwise).

5 Fluorescence Microscopy

All experiments were performed using an Axio Observer Z1 microscope (Carl Zeiss), equipped with a plexiglass incubator (Pecan), temperature and CO₂ control (Pecan), and Hamamatsu Orca CCD camera. Microscope and camera were controlled via
 10 AxioVision software (Carl Zeiss).

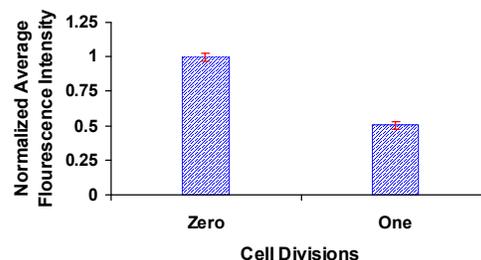


Figure 4: Quantifying CFSE fluorescence. The majority of cell divisions observed result in daughter cells with roughly half the fluorescence intensity as compared to the parent cell. The graph was generated from analysis of 20 separate cell division events. The error bars represent one standard error of the mean.

Division Symmetry Assay

In order to track cell division, CD34⁺ cells were stained with
 15 1 μM carboxyfluorescein succinimidyl ester (CFSE) as previously described.³ Alternatively, cells were incubated in 100 nM tetramethyl rhodamine methyl ester (TMRM) at 37°C. In all cases they were allowed to rest overnight prior to assay. Bright field and CFSE fluorescence images were obtained and analyzed
 20 using Axiovision software (Carl Zeiss).

Apoptosis Assay

Prior to loading into devices, cells were first incubated with 10 μM Fluo-3 (Invitrogen) for 30 minutes at 37°C. Cells were then
 25 perfused with a solution containing 5 μg/ml propidium iodide (Invitrogen), 15 μl ml⁻¹ Annexin V (Alexa Fluor 647 conjugate, Invitrogen), and 1 mM CaCl₂ (Fisher Scientific) in sterile cell culture media. In addition, for experimental samples 150 nM dasatinib (Bristol-Myers Squibb) was included to induce
 30 apoptosis. Finally, in appropriate experiments, 10 μl ml⁻¹ Hoechst dye was added to the perfusing media to label nuclei.

Results and Discussion

Monitoring Stem Cell Division

The ability to observe stem cell division and proliferation is
 35 essential for two reasons: First, with regard to platform validation, cell division is unlikely to proceed if cells are not in a suitable niche with the necessary nutrients. Continual cell proliferation indicates that the microfluidic environment is well-equipped to support long-term cell culture. Indeed, as shown in
 40 Figures 3A and B, both normal and CML stem cells were observed to proliferate whilst trapped in the device (see ESI material for movie). Viability studies were carried out at different flow rates. The optimal flow rate was found to be between 50 and 80 nl min⁻¹ (data not shown), with the later
 45 corresponding to an average linear velocity of 4 mm min⁻¹.

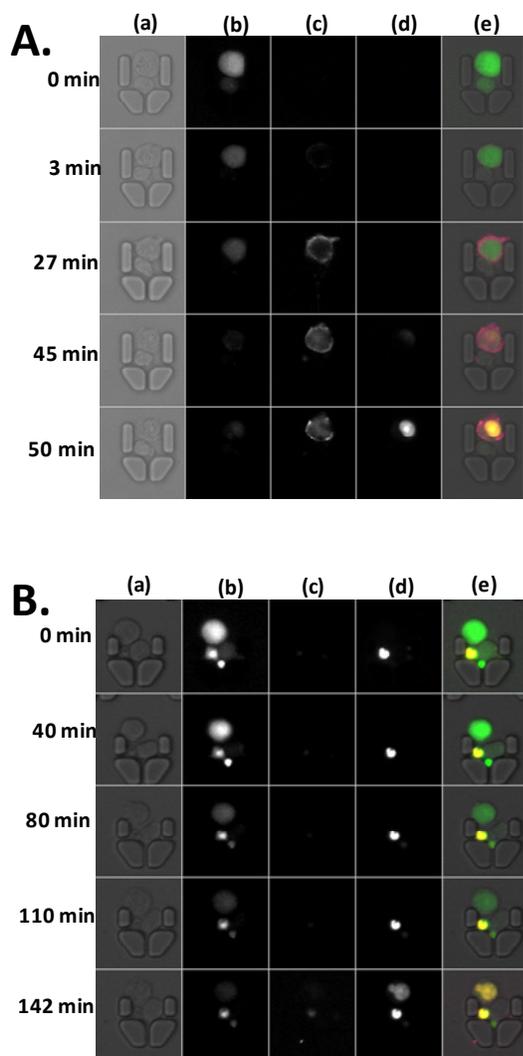


Figure 5: Imaging cell death. Normal (A) and CML (B) CD34⁺ HSCs were exposed to 150 nM Dasatinib to induce apoptosis. Cells were labelled with 10 μM Fluo-3 calcium indicator (lane b) prior to loading. Cell media perfusing device was supplemented with 15 $\mu\text{l ml}^{-1}$ Annexin-V Alexa-fluor 647 conjugate (lane c) and 5 $\mu\text{g ml}^{-1}$ propidium iodide (lane d). Combinations of the bright field (lane a) and fluorescence images (b-d) are shown in (lane e). In those cell which apoptose, normal CD34⁺ stem/progenitor cells initiate apoptosis on a faster time scale compared to their CML counterparts. For both (A) and (B) we have selected trap elements within the array that contain two cells in order to show contrast between cell death and surviving cells. In each case one cell is viable, whilst the second cell has been killed.

Secondly, in the context of basic science, stem cells are known to be unique in their ability to both self-renew and differentiate into more specialized cell phenotypes. It is hypothesized that HSCs have the ability to undergo symmetric self-renewal divisions (increase in stem cell pool, daughter cells are both HSC), asymmetric cell division (maintenance of stem cell pool; one daughter HSC and one differentiating daughter cell) and

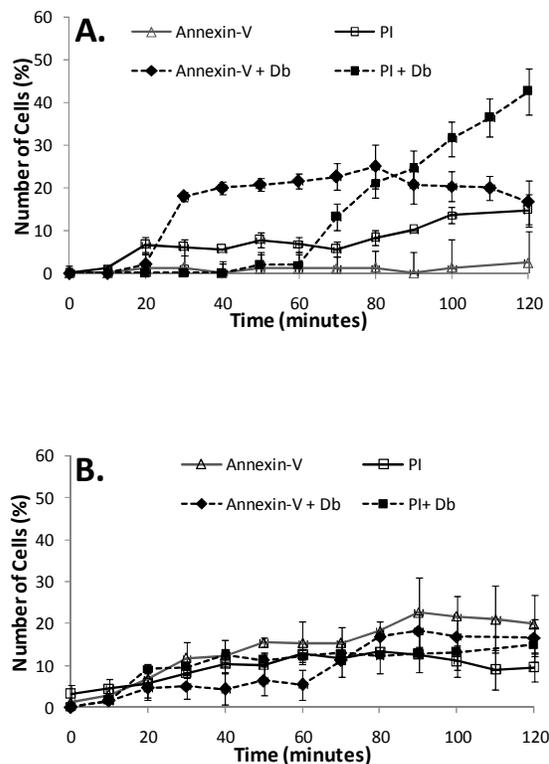


Figure 6: Dynamic viability assay. In graphs (A) and (B) are the quantified results of normal and CML stem cell fluorescent staining patterns, respectively, following exposure to 150 nM dasatinib within the microfluidic device. In (A), a clear increase in both Annexin-V and propidium iodide staining (Annexin-V +Db and PI + Db, respectively) was observed in normal CD34⁺ stem/progenitor cells exposed to dasatinib (closed symbols) relative to controls (open symbols). This indicated an increase in cell apoptosis/necrosis in the presence of dasatinib. Alternatively, in (B), over the same time scale, CML stem cells showed no distinguishable increase in staining for dasatinib over controls. This indicated no increase in apoptosis/cell death rate in cells exposed to dasatinib relative to controls. It is likely that an increase in apoptosis would be observed over a longer time scale. However, these cells are known to be resistant to chemotherapeutic intervention and this contrast in early response to drug compared to normal cells warrants further investigation. 275 CML cell in control (n=2), 284 CML cells in drug (n=4), 255 normal cells in drug (n=3), 294 normal cells in control (n=3). Error bars reflect one standard deviation about

symmetric differentiation division (reduction of stem cell numbers, two differentiating daughter cells).^{10, 11} In Figure 3A and B, the fluorescent markers TMRM and CFSE were used to label mitochondria and cytoskeletal elements, respectively. In both cases, examining the distribution of dye during cell division revealed a symmetric partitioning of cell structure amongst daughter cells. TMRM photobleached quickly and thus it was difficult to quantify, based on fluorescence intensity alone.¹⁵ However, in it is clear that mitochondria polarize during mitosis in such a way that approximately half remain in each daughter cell. Analysis of fluorescence intensity measurements of the highly-stable CFSE dye has been shown as a reliable means to monitor cell division.¹² As expected, fluorescence intensity of

CFSE in CD34+ HSCs halves with each division within the device as shown in Figure 4. Future studies will explore the

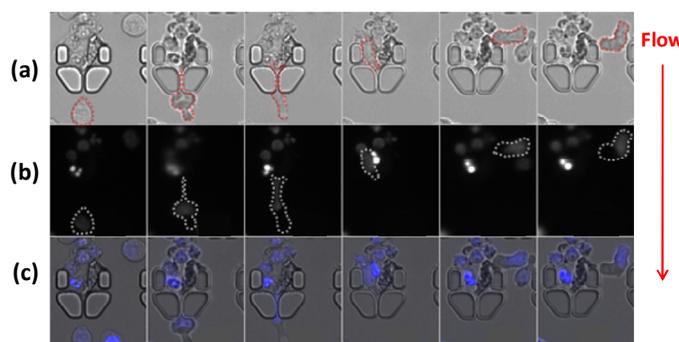


Figure 7: Imaging cell migration. Image series illustrating the active migration of CML stem cell upstream through a 2 μm gap and opposing 80 nl min^{-1} fluid flow rate (arrow indicates direction of flow). Bright field images shown in (row a), Hoechst fluorescence labelling cell nuclei shown in (row b), and combined images shown in (row c). Outlines in (a) and (b) demark the cell which is migrating upstream. Under normal conditions these cells are observed to be in constant motion and often actively migrate out of traps through the small gaps.

effects of manipulating the fluidic environment upon division symmetry using indicators such as CD34 and Numb to detect cell differentiation and asymmetric cell division.

Dynamic Viability Assay

Dasatinib inhibits proliferation and induces apoptosis in CML progenitor and terminally differentiated cells. However, the quiescent CML stem cell population is resistant to the apoptotic effect of dasatinib.³ To better understand how some cells are able to escape dasatinib-induced cell death, we must analyze individual cells and identify mechanisms which allow for survival.

The microfluidic device described provides an ideal platform on which to perform parallel, high-throughput, high content drug analysis in populations of single CD34+ stem/progenitor cells. Figures 5A and 5B show normal and CML cells, respectively, dynamically labelled with Annexin-V (lane c) and propidium iodide (lane d) within the device whilst exposed to 150 nM dasatinib in solution. As expected, normal cells undergo apoptosis as demonstrated by the transition from Annexin-V staining to propidium iodide (See ESI material for corresponding movie). After approximately 60 minutes, cells begin to die rapidly as shown in Figure 6A, such that nearly 50% stained positive for propidium iodide after two hours. Whilst the results are consistent with that observed in other studies,¹³ these results demonstrate a more rapid transition to cell death within the device as compared to conventional culture techniques. This may be due to fluidic nature of the device which delivers drugs and nutrients to the trapped cells at a significant flow rate rather than relying on diffusion in static culture configurations. The drug delivery time scale *in vivo* may lie somewhere between the diffusion limited mass transfer of static culture and the flow driven delivery of the microfluidic device. In addition, because of the continuous perfusion, the cells in the device may be more active metabolically than cells immersed in a stagnant cell culture dish.

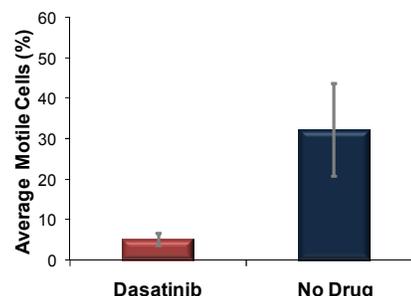


Figure 8: Dasatinib reduces cell motility. Upon exposure to 150 nM dasatinib, migratory behavior was markedly reduced as shown in here. Motile cells are those which were observed to actively move out of the traps (clearly opposing fluid flow), not including cells which exhibit random movement within the traps. Only 5% of cells exposed to dasatinib actively migrated outside of the traps compared to 30% of cells in media without drug. Student's t-test indicates migratory behaviour of dasatinib exposed population is statistically different to controls with a p-value of 0.9983. Whilst CML cells do not rapidly undergo apoptosis in response to dasatinib, the presence of the drug clearly interrupts normal migratory activity.

The cell viability data for CML cells exposed to dasatinib are indistinguishable from those of control populations, as shown in Figure 6B. Cells that do die are only stained with propidium iodide and not Annexin-V, indicating that these cells do not undergo apoptosis in response to dasatinib. This apparent resistance to drug effects might be attributed to additional activated survival pathways in the leukemic cells rendering these cells less vulnerable to the effects of the drug. These results suggest that TKI naive CD34+ CML stem/progenitor cells are resistant to the effects of dasatinib from first exposure. Furthermore, this insensitivity to drug is not a consequence of prolonged drug exposure inducing the development of resistance mutations through competitive selection. The exact mechanisms which promote this TKI resistance in CML stem cell fractions remain, however, unknown.

Analyzing Cell Motility Characteristics

Observation of cells within the device under control conditions revealed that CML cells are highly motile and can exert large mechanical forces in order to migrate against fluid flow (as high as 80 nl min^{-1}) and squeeze through gaps as small as 2 μm in width. Figure 7 shows a single CD34+ CML stem/progenitor cell (nuclei stained with Hoechst) migrating upstream through the 2 μm gaps in the cell traps (See ESI material for corresponding movie). It is possible that the cells are responding to products secreted by cells upstream, or that shear stress exerted by flow activates a migratory mechanism within the cells. Regardless of the reason, the presence of dasatinib markedly reduces cell motility in CML cells. As shown in Figure 8, only five percent of CD34+ CML stem/progenitor cells exposed to dasatinib exhibited highly migratory behavior, defined as actively migrating out of a PDMS trap against fluid flow (not including random movements within the trap), compared to greater than 30% of cells in the absence of drug.

Dasatinib is a potent, dual Abl/Src kinase inhibitor and src kinases are known to play a role in actin organization and enhancing cell motility.^{14, 15} Studies have shown that dasatinib

specifically interrupts cell motility in other forms of cancer including prostate, melanoma, and Ewing sarcoma.¹⁶⁻¹⁹ It is likely that a similar process occurs in CML cells; a subject currently under investigation. Whilst these cells are resistant to apoptosis, dasatinib does exert a significant inhibitory effect upon cell motility by blocking src kinase mediated activity. One hypothesis is that the fraction CD34+ CML stem/progenitor cells whose migratory behavior is left uninhibited by dasatinib is capable of migrating to HSC niches within the bone marrow where, in the hypoxic microenvironment, they become deeply quiescent and resistant to the effects of the drug. These cells would then account for the persistent molecular disease and rapid relapse upon drug therapy withdrawal.

Conclusions

This study utilizes a microfluidic platform to analyse hundreds of single cells in parallel. Previously, we demonstrated that this platform enables detection of paracrine signaling in human immune cells⁴ and allows quantification of the effects of toxins upon cell mechanical activity²⁰. We now show that this platform is well suited for the study of patient-derived CD34+ hematopoietic stem/progenitor cells and their response to dasatinib. Successful on-chip implementation of dynamic cell proliferation, viability, and motility, techniques which are otherwise difficult to perform using conventional methods, was demonstrated. Results indicate that patient-derived stem cells (both normal and CML) proliferate as normal in the constant flow-through environment. Dynamic, live cell, on-chip viability assays revealed that CML stem cells exhibit increased resistance to cell death upon exposure to dasatinib. However, despite this resistance, dasatinib clearly reduced cell migratory behaviors of CML cells within the microfluidic device which indicate the drug is interrupting src kinase mediated pathways.

Acknowledgements

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Electronic Supplementary Information (ESI)

Movie 1: Cell loading. This short video demonstrates the passive nature of cell loading within the device. Images were captured at maximum camera acquisition rate for two minutes. At flow rates not exceeding 80 nl min⁻¹, loading of cell traps is effective, rapid, and well tolerated by cells. (File name: cell loading.avi)

Movie 2: Monitoring cell division. This movie corresponds to still frames shown in Figure 3A illustrating cell division within the microfluidic device. Cells were preloaded with TMRM to label mitochondria. Images (brightfield and fluorescence) were acquired every 30 seconds for 45 minutes. (File name: Cell division_TMRM)

Movie 3: Imaging cell death. This movie corresponds to still frames shown in Figure 5A illustrating cell normal stem/progenitor cell apoptosis in response to 150 nM dasatinib exposure. Cells were preloaded with Fluo-3 AM Ester (green) prior to experiment. Annexin-V (pink) and propidium iodide (orange) were delivered along with the dasatinib in the media perfusing the device at a flow rate of 80 nl min⁻¹. Images were acquired every minutes for two hours. (File name: apoptosis.avi)

Movie 4: Cell migration. This movie corresponds to the still frames shown in Figure 7. Here we observe a cell, labeled with Hoechst dye (blue) delivered by perfusing media, migrate upstream against an 80 nl min⁻¹ flow rate whilst squeezing through 2µm gaps between PDMS structures. Dead cells are labeled with propidium iodide (orange). Images were acquired every minute for five hours. (File name: cell migration.avi)

References

1. T. G. Lugo, A. M. Pendergast, A. J. Muller and O. N. Witte, *Science*, 1990, **247**, 1079-1082.
2. J. D. Rowley, *Nature*, 1973, **243**, 290-293.
3. M. Copland, A. Hamilton, L. J. Elrick, J. W. Baird, E. K. Allan, N. Jordanides, M. Barow, J. C. Mountford and T. L. Holyoake, *Blood*, 2006, **107**, 4532-4539.
4. S. Faley, K. Seale, J. Hughey, D. K. Schaffer, S. VanCompernelle, B. McKinney, F. Baudenbacher, D. Unutmaz and J. P. Wikswo, *Lab Chip*, 2008, **8**, 1700-1712.
5. A. Prokop, Z. Prokop, D. Schaffer, E. Kozlov, J. Wikswo, D. Cliffel and F. Baudenbacher, *Biomed Microdevices*, 2004, **6**, 325-339.
6. K. Kamei, *Lab Chip*, 2009.
7. A. Valero, J. N. Post, J. W. van Nieuwkastele, P. M. Ter Braak, W. Kruijer and A. van den Berg, *Lab Chip*, 2008, **8**, 62-67.
8. G. H. Underhill and S. N. Bhatia, *Curr Opin Chem Biol*, 2007, **11**, 357-366.
9. N. Korin, A. Bransky, U. Dinnar and S. Levenberg, *Biomed Microdevices*, 2008.
10. A. D. Ho and W. Wagner, *Curr Opin Hematol*, 2007, **14**, 330-336.
11. A. Marciniak-Czochra, T. Stiehl, A. D. Ho, W. Jager and W. Wagner, *Stem Cells Dev*, 2008.
12. A. B. Lyons, *Immunol Cell Biol*, 1999, **77**, 509-515.
13. M. Copland, F. Pellicano, L. Richmond, E. K. Allan, A. Hamilton, F. Y. Lee, R. Weinmann and T. L. Holyoake, *Blood*, 2008, **111**, 2843-2853.
14. R. Guiet, R. Poincloux, J. Castandet, L. Marois, A. Labrousse, V. Le Cabec and I. Maridonneau-Parini, *European Journal of Cell Biology*, 2008, **87**, 527-542.
15. S. Hiscox, N. Jordan, L. Morgan, T. Green and R. Nicholson, *Clinical and Experimental Metastasis*, 2007, **24**, 157-167.
16. F. Timeus, N. Crescenzo, A. Fandi, A. Doria, L. Foglia and L. Cordero di Montezemolo, *Oncol Rep*, 2008, **19**, 353-359.
17. S. I. Park, J. Zhang, K. A. Phillips, J. C. Araujo, A. M. Najjar, A. Y. Volgin, J. G. Gelovani, S.-J. Kim, Z. Wang and G. E. Gallick, *Cancer Res*, 2008, **68**, 3323-3333.
18. A. C. Shor, E. A. Keschman, F. Y. Lee, C. Muro-Cacho, G. D. Letson, J. C. Trent, W. J. Pledger and R. Jove, *Cancer Res*, 2007, **67**, 2800-2808.
19. R. Buettner, T. Mesa, A. Vultur, F. Lee and R. Jove, *Mol Cancer Res*, 2008, **6**, 1766-1774.
20. E. Kim, Sivasubramaniam, Priya, Wikswo, John P., Seale, Kevin T., *mTAS International Conference on Miniaturized Systems for Chemistry and Life*, 2008, **12**.