Lab on a Chip

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Lab-on-DVD for HIV diagnostics

Two lab-on-a-chip characteristics – price and disposability – hold the most attractive potential in the context of global healthcare applications. While point-of-care (POC) diagnostic devices are considered as a convenient and affordable alternative to in-patient visits in developed countries, in the developing world, these devices are crucial for early diagnosis and reduction in the spread of diseases. For example, HIV ranks at the top of the list of debilitating, transmittable diseases,[1] and early diagnostics and monitoring the progress of this disease is of utmost importance. In the attempt to target these issues, portable and low-cost lab-on-a-chip platforms and POC devices are needed, and are currently being pursued in bioengineering research.[1,2]

During HIV infection, the virus targets a specific lymphocyte: T-helper cells, which contain a cluster of differentiation 4 (CD4+) at its surface. The number of CD4+ T-cells in the blood of healthy adults ranges from ~800–1000 cells μL^{-1} and the reduction of these cells is among the major diagnostic tests for HIV progression. When the number of CD4+ cells falls below 200 cells μL^{-1}, drug cocktails need to be administered to protect patients against other infections. The gold standard for monitoring CD4+ count requires a flow cytometer, which is expensive, requires trained personnel and is not available in resource-scarce settings.

The 'Lab-on-a-CD' platforms are low-cost solutions for point-of-care devices and have found numerous applications in biomedical devices, including sample preparation, and PCR amplification.[2] Furthermore, the difficulty in integrating detection systems into these rotating platforms has limited their widespread use. In this context, Russom and colleagues created a 'lab-on-a-DVD', which – based on a standard DVD computer drive – is capable of handling and testing blood samples for HIV. Ramachandraiah *et al.*[3] built a DVD-based laser scanning microscope (DVD-LSM), which combined the rotational control of the DVD for liquid handling with an integrated image-based assay for HIV diagnostics.

Ramachandraiah *et al.*[3] modified a standard DVD drive by placing a second photodetector (photodiode array) above the DVD (Fig. 1A). This provided an ability to image particulates on the surface of the DVD, by tracking changes in the absorbance and light scattering from the light emitted by the DVD laser. Additionally, the photodetector collected data and combined the information into a final image with a resolution down to 1 μm. The complete setup also included a rotational stage (for liquid handling) and a temperature controller, to maintain constant temperature. The microfluidic DVD device consisted of two-half disc substrates, where the bottom substrate contained all the operational information required to read the disc using a standard DVD drive and the top half-disc substrate contained the fluidic microchannels, access through-holes, and other fluidic reservoirs. After surface modification, CD4 antibodies were deposited into the fluidic channels, and after perfusing whole blood into the channels, the authors were able to capture and image the CD4+ cells (Fig. 1B). However, low capture efficiency (only 50%) suggests

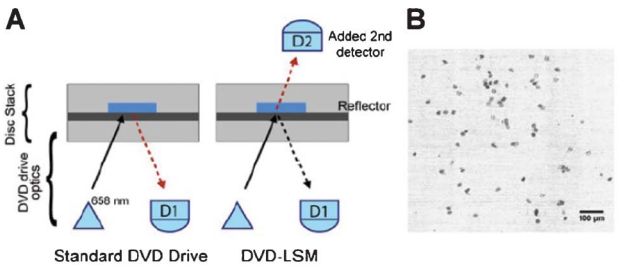

Fig. 1 (A) A comparison between a standard DVD drive and the DVD-Laser Scanning Microscope (DVD-LSM) mode of operation. In the DVD-LSM a second detector (D2) was placed above the disc. (B) CD4+ cells captured on the surface of the DVD coated with anti-CD4 antibodies, using the DVD-LSM. Adapted from Ramachandraiah *et al.*[3]

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that further improvements are required, either by optimizing the geometry of the channels or by improving the binding efficiency. Other microfluidic approaches for HIV diagnostics for CD4+ cell counting, included a charge-coupled device (CCD) camera.[4] However, the CCD camera approach had several shortcomings compared to the 'lab-on-a-DVD' system, since it used an expensive CCD camera and lacked the ability for automated liquid handling.

Affordability is a key feature in the presented application. By configuring a standard DVD drive to function as a liquid handling system with temperature control, and integrating it with a photodiode-based detection system, Ramachandraiah and his colleagues have created a platform that can be used for numerous biomedical applications. In the absence of flow cytometers, the low cost, effective and easy to use DVD-LSM system makes it a viable global health solution for POC diagnostic devices, especially for developing countries. Further improvements could transform the platform into a portable and inexpensive cell sorting cytometer. Alternatively, it could be applied for affordable disease diagnostics in developing countries and in scenarios where both lack of resources and fast response times are required.

Atherosclerosis from different angles

Atherosclerosis, the primary cause of coronary heart disease, affects a large part of the world's population. Several existing disease models satisfactorily mimic different stages or features of atherosclerosis.[5] However, thrombus formation and plaque rupture, key problems in the disease's development, are not yet fully understood.[6] Briefly, atherosclerosis can lead to a narrowing of arterial vessel diameter – stenosis – by the accumulation of fatty material (such as cholesterol) in the arterial wall and atherosclerotic foam cell formation. Stenotic features of the arterial walls (Fig. 2) can originate from thrombus formation by accumulation of platelets on the arterial walls. *In vitro* studies of this process cannot be easily carried out on standard static cell culture platforms, justifying the need for improved models.

Motivated by this challenge, Heemskerk and colleagues[7] developed a microfluidic system to study the influence of stenosis geometry on thrombus formation under controlled flow conditions. Specifically, Westein *et al.*[7] designed a reconfigurable microfluidic platform where the channel restrictions ranged from 20–80%. Perfusing the channels with blood allowed the unveiling of key parameters on the post-stenotic platelet aggregation (outlet, Fig. 2) and its relation to stenosis geometry.

First, computational fluid dynamics analysis of the stenosis percentage (20–80%) revealed that higher degrees of stenosis caused an increase in shear stress. Then, the authors covered the surface of the channels with patches of von Willebrand factor (vWF) and fibrinogen, both involved in the process of platelet aggregation in thrombotic sites. Perfusing blood into these channels demonstrated an increasing platelet aggregation with higher degrees of stenosis, while addition of iloprost (a platelet inhibitor) prevented that increase. Also, as the wall shear stress rates increased (600, 1000 and 2000 s^{-1}) so did the platelet aggregation. A microfluidic approach of a recent study on microvascular occlusion and thrombosis[8] indicated a link between vWF and platelet aggregation, by showing that platelet aggregation was co-localized with vWF expression sites. Here, Westein concentrated on both the geometrical cues and vWF to the contribution for platelet aggregation in stenotic regions. Accordingly, the microfluidic channels were coated with an endothelial cell monolayer and perfused with buffer or blood. Perfusion with only buffer at a wall shear stress of 1000 s^{-1} led to surface expression of vWF in the post-stenotic region (Fig. 2, outlet), while in straight channels vWF expression remained constant, even with higher wall shear stress rates. When perfusing whole blood, large platelet aggregates were found to localize in the post-stenotic site (Fig. 2, outlet), while smaller aggregates were found in the pre-stenotic site (Fig. 2, apex).

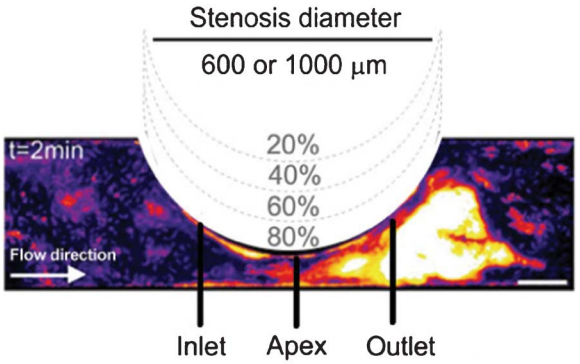

Fig. 2 A confocal image of the microfluidic channel, with 80% stenosis, coated with endothelial cells and perfused with blood. Platelet aggregates can be seen in the apex and outlet regions of the stenosis (in red and yellow). Different amounts of stenosis (in grey) illustrate the range in channel reductions (20–80%) and the stenosis diameter used in this model. Adapted from Westein *et al.*[7]

Stenotic geometries are intrinsically correlated with disturbances in hemodynamic flow, and consequently have biological effects. They contribute to increased expression of vWF by endothelial cells when perfused, which will play a role in platelet aggregation. The work by Westein *et al.* has helped gain insight on the influence of distinct geometrical cues on thrombus formation and will ultimately result in tools to better understand the atherosclerotic disease process. It highlights the importance of flow and geometry in vascular research, and the need for models that better mimic these features. Moreover, it also contributes to the knowledge on the role and biology of vWF in platelet aggregation, consolidating this multimeric protein as a biomarker for atherosclerosis.

Working out new avenues to repair muscle

Routine exercise and small injuries produce tears and damage in skeletal muscle, which activate satellite cells – the resident stem cell population of the muscle – to repair and develop the tissue. The mononucleated satellite cells migrate to the damaged muscle, proliferate, and then fuse to form multi-nucleated myotubes that integrate with the local tissue.[9] In this manner, muscles possess a large capacity for growth and repair, allowing the muscles of our bodies to bulk up after a few weeks in the gym and heal quickly after running a marathon. However, severe trauma to skeletal muscle often leads to incomplete repair and loss of function as fibrotic tissue forms in place of functional muscle. Thus, strategies to direct or encourage the regeneration process are needed to treat acute muscle injuries. While the basic process of satellite cell repair is well-defined, the manner by which satellite cells and myoblasts (skeletal muscle progenitor cells) interact with other resident cells of the muscle niche and receive signals from the extracellular matrix during repair is not fully understood. These complex interactions, namely myoblast and fibroblast interactions, are likely involved in fibrosis and incomplete repair of acute muscle damage.

To better characterize the inter-relationship between cells of the muscle niche, Rao *et al.* have recently employed a co-culture microdevice to study contact-dependent and contact-independent communication between murine fibroblasts and murine myoblasts.[10] Specifically, the authors adapted a reconfigurable, co-culture chip developed by Bhatia and colleagues,[11] which resembles interlocking combs that allowed them to culture myoblasts or fibroblasts on the fingers of each comb. By interlacing the combs, myoblasts were cultured in direct contact with populations of other myoblasts or fibroblasts or cultured with an 80 μm gap between cell populations (Fig. 3). In this setup, the authors investigated the effect of co-culture with fibroblasts on myoblast alignment and differentiation into myotubes, both critical parameters in muscle repair, and the extent to which these effects were mediated by paracrine or contact dependent signals. These studies revealed that fibroblasts increased the alignment of neighboring myoblasts, while decreasing their differentiation efficiency.

The surfaces of the silicon comb fingers of the co-culture chip were initially coated with collagen (1 mg mL^{-1}) to deposit an adhesive protein on the surface. Subsequently, a confluent layer of 3T3 fibroblasts or C2C12 myoblasts were grown on separate combs and pairs of combs were interlaced, with and without a gap of 80 μm. The devices were cultured for 6 days prior to fixing and staining for cell markers. Myosin heavy chain, a marker for myotube differentiation, was used to quantify myoblast differentiation and alignment with fluorescence microscopy. When cultured in direct contact, fibroblasts increased the average alignment of the myoblasts relative to the edge of the finger of the comb by approximately 10°. This effect, however, was not observed when myoblasts were co-cultured with fibroblasts with an 80 μm gap between the two

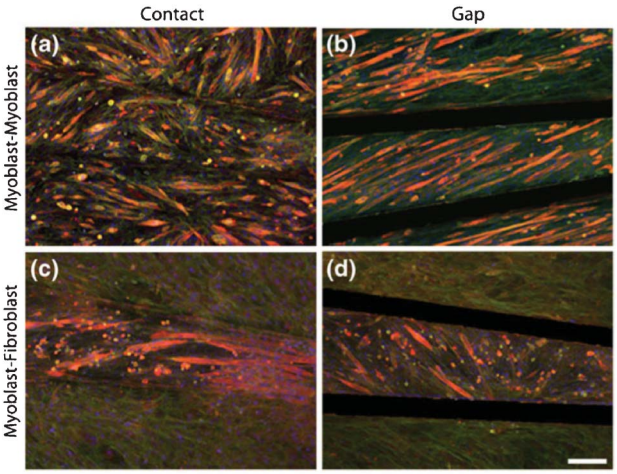

Fig. 3 Representative fluorescence images of co-cultures after staining. Nuclei, f-actin, and myosin heavy chain are shown in blue, green, and red, respectively. (A) Myoblast–myoblast in contact. (B) Myoblast–myoblast with 80 μm gap. (C) Myoblast–fibroblast in contact. (D) Myoblast–fibroblast with 80 μm gap. Scale bar, 200 μm. Figure reprinted with permission from Rao *et al.*[10]

cell populations, indicating that increased alignment was a contact-dependent phenomenon. In the same experiments, both gap and contact myoblast–fibroblast co-cultures indicated a significant decrease in myotube differentiation (~50% more nuclei co-stained for myosin heavy chain in the myoblast–myoblast co-cultures). These experiments were extended to demonstrate that alignment and differentiation effects in the co-culture were communicated by fibroblast growth factor (FGF-2), as the addition of a neutralizing antibody against FGF-2 abrogated the effects. Furthermore, the co-culture effects were shown to require bidirectional signaling or cross-talk between the cell types since fibroblast conditioned media alone did not replicate the effects on myoblast differentiation.

In total, these experiments highlight the importance of cell–cell interactions on the functionality of the cells of the muscle niche. While this work does not fully elucidate the mechanisms of the cross-talk between murine myoblasts and fibroblasts, it does suggest that contact and FGF-2 signaling are important in regulating myoblast function. Ultimately, these findings suggest new mechanisms to increase the alignment and to control the differentiation of muscle cells, which should provide improved avenues for the repair of damaged muscle *in vivo*.

References

1 C. D. Chin, V. Linder and S. K. Sia, *Lab Chip*, 2007, **7**, 41–57.
2 M. Madou, J. Zoval, G. Jia, H. Kido, J. Kim and N. Kim, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 601–628.
3 H. Ramachandraiah, M. Amasia, J. Cole, P. Sheard, S. Pickhaver, C. Walker, V. Wirta, P. Lexow, R. Lione and A. Russom, *Lab Chip*, 2013, **13**, 1578–1585.

4 S. Moon, H. O. Keles, A. Ozcan, A. Khademhosseini, E. Haeggstrom, D. Kuritzkes and U. Demirci, *Biosens. Bioelectron.*, 2009, **24**, 3208–3214.
5 V. Singh, R. L. Tiwari, M. Dikshit and M. K. Barthwal, *Curr. Vasc. Pharmacol.*, 2009, **7**, 75–109.
6 C. K. Glass and J. L. Witztum, *Cell*, 2001, **104**, 503–516.
7 E. Westein, A. D. van der Meer, M. J. Kuijpers, J. P. Frimat, A. van den Berg and J. W. Heemskerk, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 1357–1362.
8 M. Tsai, A. Kita, J. Leach, R. Rounsevell, J. N. Huang, J. Moake, R. E. Ware, D. A. Fletcher and W. A. Lam, *J. Clin. Invest.*, 2012, **122**, 408–418.
9 A. D. Bach, J. P. Beier, J. Stern-Staeter and R. E. Horch, *J. Cell. Mol. Med.*, 2004, **8**, 413–422.
10 N. Rao, S. Evans, D. Stewart, K. H. Spencer, F. Sheikh, E. E. Hui and K. L. Christman, *Biomed. Microdevices*, 2013, **15**, 161–169.
11 E. E. Hui and S. N. Bhatia, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 5722–5726.