On mixing people and ideas - a brief history of the TopoChip

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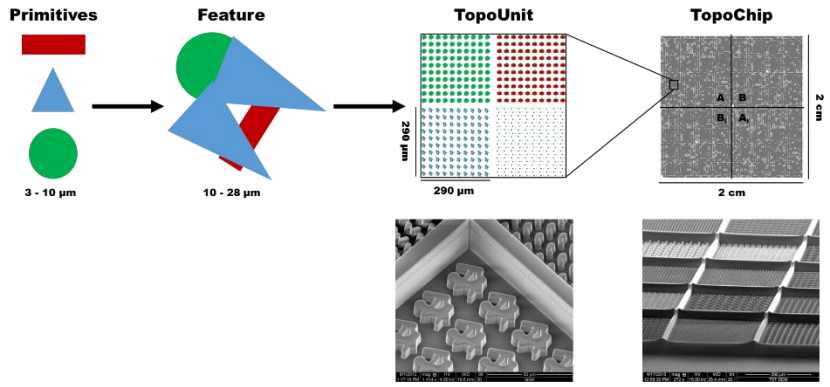
Do you wonder who invented the technology that allows you to read the text on this screen? Who invented the transistor which is at the basis of the memory in your device, who came up with the idea to use bits to code information and who came up with the technology for flat screens? Was it an isolated individual, a Gregor Mendel growing peas in a monastery garden, or was it a team of people each making their own little contribution, as in sequencing of the human genome. True, Fred Sanger invented DNA sequencing but who wrote the scripts to align the DNA sequences?

I wrote this assay, in the shape of a timeline, to sketch a picture of the birth of the TopoChip, a high throughput screening platform for biomaterial surface topography. With it I hope to show the process leading to new technology. I may update this brief history at some point when new facts float up or when my memory turns out to be incorrect.

The TopoChip

So, what are we talking about? The current TopoChip design comprises a 2x2 cm chip, divided in 66 rows and 66 columns of 290x290 micron squares (refered to as TopoUnits). Each TopoUnit is surrounded by a 50 micron high wall and the bottom of each unit is patterned with 10 micron high pillars. The x-y dimensions of the pillars are produced by a design algorithm that uses primitives: circles, triangles and rectangles. The algorithm puts random primitives at random spots in an area of a specified size. The resulting design is referred to as a feature. When we use integers for the design parameters, we can build a library of 150 million different features. The chips are produced using photolithography in silicon and subsequent stamping (embossing) into a polymer of interest. The chip can be seeded with cells, which can then be analysed using imaging. Based on the choice of polymer, cell type and molecular marker, the chip can be used to screen for optimization of any medical device or cell culture platform, or to investigate cellular responses to micropatterns.



Some people involved

Myself, *Jan de Boer*, geneticist and cell biologist. With a background in mouse genetics, I performed genetic modifier screens in *Drosophila melanogaster* a.k.a the fruitfly from 1999 to 2001 in Cambridge, UK as a postdoc. I started working on bone tissue engineering at the biotech company IsoTis S.A. in 2002. Initially I focused on Wnt signaling in mesenchymal stem cells, but later extended this to other signaling cascades and the use of embryonic stem cells. My first experience with biomaterials was an attempt to controlled release of the Wnt mimic lithium from calcium phosphate ceramics in 2003. Later, I started using 3D porous ceramics as scaffold for tissue engineering and even later to grow cartilaginous grafts using embryonic stem cells.

Clemens van Blitterswijk: Clemens is a cell biologist from training but he extensively collaborated with pioneers in the field of biomaterial engineering to help shape the field of tissue engineering, first at Leiden University, later at the company IsoTis S.A., founded by himself and by calcium phosphate pioneer Klaas de Groot. Since the mid 1990's, IsoTis was at the forefront of many developments in the field of tissue engineering. For instance, the Netherlands' first 3D bioprinter was stationed here, polymer-based controlled drug release systems were developed as well as bone inducing ceramics, which were later commercialized by Progentix. Clinical trials were conducted in bone, skin and cartilage tissue engineering. Clemens' and Klaas' free spirits were reflected in the IsoTis R&D department as a place where everything was possible, from printing ears, growing human embryonic stem cells to using shell proteins for biomaterial engineering.

Dimitrios Stamatialis, is a biomaterials engineer at the department of Membrane Technology at the University of Twente with a background in membrane technology and focus on separating molecules using membranes. Dimitrios has a keen interest in biological applications of membranes and was at the time one of a small group of people at Twente University to combine cell biology and material engineering.

Roman Truckenmüller is an engineer who led a team on microfabrication and microfluidics at Karlsruhe Institute of Technology in Karlsruhe, Germany. Roman pioneers the soft embossing technique in which he imprints structures into polymer films without disrupting the polymeric architecture.

Marcel Reinders is a computational scientist at the TU Delft, with background in image recognition, but he made his marks in the early days of gene expression analysis. Marcel and I met in a joint research project between IsoTis and pharmaceutical company Organon. The interaction is mostly on gene expression studies performed by Organon within this study, later on other gene expression studies in my lab.

December 2003

Clemens and I change affiliation from biotech company IsoTis to Twente University, but we stay at the IsoTis laboratories in Bilthoven, the Netherlands until September 2006. Clemens steps down as CEO and refocuses on science.

2004

The “summer of love” for materials engineering, at least in perception. Dan Anderson publishes his seminal paper on high throughput screening of libraries of biomaterials and Chris Chen his on the effect of cell shape on osteogenic and adipogenic differentiation of mesenchymal stem cells. Clemens and I discuss the content of these papers, which have a huge impact on the way we see tissue engineering and biomaterial engineering. We elaborate on the possibility to implement high throughput screening on biomaterials which are then used at IsoTis: calcium phosphate ceramics and block co-polymers. I had some discussions with Joost de Wijn on combinatorial chemistry with the PolyActive block co-polymer platform which was developed in house, and with Florence Barrere, a calcium phosphate expert from the school of Klaas de Groot, with whom I shared an office. She engineers calcium phosphate coatings and we discuss the possibility to use 96 well plates to increase throughput of coating screening. Florence writes, and is granted, a European project. Hemant Unadkat starts on this project as a PhD student, until Florence leaves the group to start a position at the newly founded company Progentix of ex-IsoTis group leader Joost de Bruin.

Laura Vogelaar, PhD student in the lab of Dimitrios Stamatialis, presents her work at the 11th Dutch annual conference on BioMedical Engineering on 4-5 October 2004 in Nationaal Sport Centrum Papendal in Arnhem, the Netherlands. Laura shows her work on micro-patterned membranes and the effect of the patterns on cellular contact guidance. Clemens attends the seminar, while I read the posters (I probably arrived late). During the coffee break, Clemens finds me, and mentions the possibility to use micro-structures to manipulate cell shape, similar to Chen’s micro-patterns, and to do this in a high throughput fashion as Dan did. We have a discussion at a poster which is presented by Laura’s master student Bernke Papenburg, where a discussion with Dimitrios starts. Minds meet, Dimitrios immediately sees the possibilities to collaborate on cell-material research. A short meeting takes place during dinner where the first microstructures are drawn on paper tissues! A follow up meeting is agreed on.

Spring 2005

Dimitrios and Bernke visit the IsoTis facilities in Bilthoven. Because there is limited access to cell culture facilities at Twente University, we gave Bernke, now a PhD student, access to the Isotis cell culture facilities. The idea of topography screening is also discussed and a “Let’s do it” is reached. Follow up brainstorms are then organized to think about topographical design space and the name TopoChip is invented (most likely by me, being a name-fetish. I also came up with the term ‘materiomics’, or to be precise ‘materialomics’ but Clemens suggested to drop the ‘al’). It was agreed that Dimitrios’ team would provide the knowledge and expertise on microfabrication, photolithography and phase separation micro-molding. The “PhD pool”, the IsoTis-based academic group of Clemens and me, would provide the infrastructure and knowledge on the biological side. In short, I would lead the biological science, Dimitrios the engineering and Bernke Papenburg did the practical work. Clemens was always available for brainstorming. Bernke’s first experiments was on surfaces patterned with lines.

Mid 2007

The PhD pool moves to Enschede in the summer 2006, with the department of Tissue Regeneration as new name. The transition speeds up the interaction between the groups.

Bernke and I set out to design the first TopoChip on paper and the 1st (manual) design was ready by June '07, with adaptions in Sep '07. We considered shapes that confine cells and impose a shape on them, ranging from circles to stars and the University of Twente logo. We also included shapes that produce pillars on which cells could sit. The shapes are organised on a chip in areas of 200x200 microns (referred to as TopoUnits) and separated by walls of 50 microns high. Bernke translates the manual design to the software package Clewin and Rob Lammertink advises how to combine the two different designs (features and walls). A mask is ordered in the MESA+ clean room, the molds were made by Rob, and Bernke casts the first TopoChips using poly lactic acid.

The first cells seeded onto the TopoChip are mouse embryonic stem cells, line E14. An iconic cell line because it was the mouse ES cell line produced by Martin Evans in which he demonstrated the possibility for gene targeting. The cell line found its way into the Netherlands through Anton Berns at the NKI and ended up in Rotterdam in the lab of Jan Hoeijmakers where I used it to target the *Xpd* gene as a PhD student. Years later, I received the cells on request from my former colleagues (an interesting case of ownership) to perform work on endochondral bone formation. Being available, E14 mouse ES cells were stained with phalloidin in November of 2007. It gave proof of principle that surfaces can be designed, produced and that cells change their shape in response to the design. As mentioned earlier, Hemant Unadkat was recruited on a European materials screening project but after his supervisor left, Clemens suggests to move him onto the TopoChip project. It is agreed that Hemant focusses on the biological aspects, Bernke on engineering. Bernke produces TopoChips using the phase separation micromoulding technique but it proves difficult to make a whole chip with robust reproduction of features and to keep the chips flat.

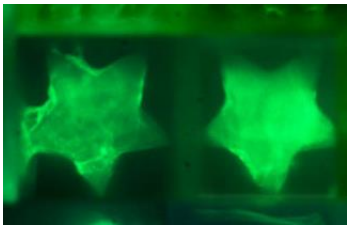


Figure 2: GFP-transfected E14 ES cells on the first generation TopoChip. It may not be the best picture, but it is one of the oldest.

Around this time, Roman Truckenmüller joins Twente University on a joint position between the department of Membrane Technology, Tissue Regeneration and the Lab on a Chip group headed by Albert van den Berg. Roman was recruited because of his extensive experience in microfabrication. Under his guidance, phase separation micromolding is abandoned as strategy to produce the chips because the sheets are not flat enough for imaging purposes. Roman suggests hot embossing instead and establishes this at the MESA+ cleanroom facilities using the Obducat machine.

2008

While the technicalities of chip production are tackled, many discussions take place on the design of the TopoChip. The TopoChip team has grown and now includes: myself, Clemens, Roman, Hemant, Bernke, Dimitrios, Gerard Post, Kamiel Cornelissen and Marc Uetz. The latter three were from the department of Discrete Mathmatics at the University of Twente, and were introduced to the team by Roman. The discussion focusses on design space: how many different shapes can we design and how different are they? Which algorithm shall we use to achieve this? During one of several discussions in the "fish bowl", the transparent meeting room of the Tissue Regeneration department, Marc Uetz rejects my idea to use the checkerboard as a design principle and reminds me of the story of King Shirham and the grains on the chess board. The sheer number of possibilities will only create noise. Roman considers the use fractals, and later realizes that the primitives comprising the fractals need not be clustered but can also be used more randomly in design space. The rationale behind the design is that circles can create large

areas, triangles can make pointy features and rectangles can make long line-shaped structures. The fishbowl is the place where the team agrees on the design and approach: Each unique design is referred to as a feature and each TopoUnit contains a repetition of one feature in the x-y axis as a 10 µm high pillar in the z direction. Three features sizes are chosen, each chip will contain replicates and a quadrant structure is chosen to have replicates on different parts of the chip. Bigger TopoUnits (290x290 um) are designed to give more cells to image.

Autumn 2008: Kamiel Cornelissen, master student in the department of Gerard and Marc, writes the algorithm for random generation of patterns. To select 2174 designs from the 150 million in silicon library, a digital code needs to be entered into the algorithm, and the story goes that Kamiel uses his birthday to produce the first series of patterns, which is used till date to produce the TopoChip. Bernke writes the Clewin design file based on Kamiel's patterns and the mask and mold are produced in the cleanroom by the local company Microknit. The TopoChips are produced by Roman in the MESA+ cleanrooms.

Hemant and I realize that the chips need to be analyzed at some point and a discussion starts at the TR department on the strategy, imaging being the most logical choice. I had a discussion with Koen Dechering of Organon, with whom I collaborated who shows me a Cellomics high content imaging microscope, a then high end machine but far too expensive for us. As an alternative, suggested by Koen, I visited a Nijmegen group who owns a DNA microarray reader, and considered per unit instead of per cell data. Meanwhile (June 2008), Hemant has discussions with Tom Groothuis of the department of Applied Physics, famous for their imaging expertise (co-inventors of the flow cytometer for instance). Tom uses open acces CellProfiler as imaging software package for his own research and suggests that it could also be used to acquire quantitative data from the TopoChip. Clemens and I decide to use part of the financial reserve of TR to purchase a high content imaging machine. Hemant, with help of Tom and Aart van Apeldoorn, set out for an intensive search, to finally choose the BD Pathway as the machine to scan the chip. The machine is purchased and scanning of the chip starts. After many online discussions with the CellProfiler service desk on how to set up pipelines for the TopoChip, Hemant stays with the Imaging platform at the Broad Institute in Boston in the summer of 2009 to learn the knitty gritty of high content imaging, image acquisition, data extraction and correction.

2010

Hemant runs two cell assays on the first polylactid acid-based TopoChips. Both use human bone marrow mesenchymal stem cells, a frequently used cell type in our skeletal tissue engineering lab. In one assay, the expression of the bone marker ALP is measured, in the other he stains the cells with BrdU, which specifically stains dividing cells. By the time he has produced the first screening data, comprising a library of tens of thousands of images, he realized that additional help was needed to handle them, which is when Marcel Reinders of the TU Delft and his PhD student Marc Hulsman come into the picture. Marcel Reinders collaborates with me on a genomics driven project with Organon, and has a background in image analysis. Based on discussion with Hemant, Marc Hulsman writes a Matlab based pipeline to acquire and clean-up the images and to extract per cell data. With the data in hand, and machine learning-based analysis by Marc, the first preparations on a manuscript are made, with a first draft of the

TopoChip manuscript in May 2010. In September 2010, the manuscript is submitted to Nature Materials but is rejected by the editor, which also happened at Nature Biotech and Nature Methods. In Januari 2011, the manuscript is submitted to PNAS and is accepted on 23 August 2011.

Since its publication, we have established both a vibrant research group and company around the TopoChip. We entered into machine learning and are now applying genetic algorithms and deep learning to find correlations between cell behavior and surface design. A new TopoChip is also born: the NanoTopoChip in which we reach primitive sizes of a few hundred nanometers. We are analyzing the molecular mechanisms using transcriptomics and gene network analysis. Some hit surfaces improve tissue interaction in animal models and are further developed for clinical application. We identified surfaces that maintain the phenotype of primary human hepatocytes and we are producing 96 well plates with this structure for drug screening purposes, our first potentially commercial product.

In retrospect

Looking back at this story there are a number of things that strike me. The first thing is time. It took six years from the conception of the idea to publication. A big chunk of time is taken by the fact that the TopoChip was only one of many parallel projects by all scientists involved. Bernke Papenburg published a handful of papers as PhD student and the TopoChip was only one of them (in fact her thesis does not include the PNAS paper but rather a chapter on the first, still unpublished TopoChip). Not until Hemant joined did we have a dedicated person on the job. Myself, I was running a reasonably sized research group, and worked on cell signaling in MSC, on transcriptomics on biomaterials, we established endochondral bone formation as a strategy for tissue engineering. TopoChip was just one of many project, as it was for everyone involved.

Another reason for delay is expertise. At the start of the TopoChip project, I had no notion of microfabrication (did not really know it existed), high content imaging or computational science. Although all this expertise was needed to run the whole project, I did not look for them in parallel. We solved problems as and when they occurred. Hot embossing instead of solvent casting, buying a microscope, writing an imaging script together with the microscopy vendor, learning to use imaging software, writing an algorithm: in each step we needed the help of experts. Often, I felt like the main contractor on the project, whereas the subcontractors did a lot of the real work. Finding people and expertise, and getting them to work just takes time.

The third thing that occurs to me is geography. Except for the Imaging platform at the Broad Institute, all scientists were at Dutch Universities or companies and of those the vast majority at the University of Twente. It is important to know that the Netherlands is a tiny country; you can reach most of it in an hour and a half of traveling. At Twente University, we were lucky to have the world's best cleanrooms at the MESA+ Nanotech Institute. The Biomedical Technology institute was led by the visionary Jan Feijen who dragged us from IsoTis S.A. in Bilthoven to Enschede to join his home-grown biomaterial community. Twente University had Mark Uetz to take on the design of patterns using his mathematical background and Roman Truckenmüller to produce them. Twente had Tom Groothuis to put us on the track of quantitative image analysis and Dimitrios Stamatialis who was a lone wolf working with cells in a

membrane technology department. As a true community of collaborative spirits and a can-do mentality, it was a great place to work.

Fourth, none of the work described in this assay was part of a TopoChip research grant. Nothing was planned, nothing budgeted, nothing reported, no bills were sent. Everyone just did it for fun and we had the freedom to do it. I was paid by IsoTis S.A. and later Twente University, Hemant was on a European grant but with another (yet flexible) scope, Bernke was funded by BMTi seed money, expenses were paid from our departmental financial reserve and so on. Interestingly, the first handful of grants that we wrote on the TopoChip were rejected and “lack of feasibility” was the main reason for rejection. As Clemens states: “Our best work is not funded by projects”.

Finally, there is memory, and the loss of it, or more precisely, the loss of parts of it. It seems that, as time progresses, some iconic events remain crystal clear (me discussing the Anderson paper with Clemens), but others have faded (who came up with the name TopoChip?). For me, it is interesting to see how many of my memories are based on mental images rather than on facts. I hope that by writing this, I have ordered the facts still available to build an image for you to capture. The image of people and ideas mixing to create something new.