Interview with Donald E Ingber

Donald E Ingber, MD, PhD, speaks to Hannah Stanwix, Managing Commissioning Editor: Donald E Ingber is the Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University (MA, USA), Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital (MA, USA), and Professor of Bioengineering at the Harvard School of Engineering and Applied Sciences (MA, USA). He received his BA, MA, MPhil, MD and PhD from Yale University (CT, USA). Dr Ingber is a founder of the emerging field of biologically inspired engineering, and at the Wyss Institute, he oversees a multifaceted effort to identify the mechanisms that living organisms use to self-assemble from molecules and cells, and to apply these design principles to develop advanced materials and devices for healthcare and to improve sustainability. In addition, Dr Ingber has made major contributions to mechanobiology, tissue engineering, angiogenesis, systems biology and nanobiotechnology. He was the first to recognize that tensegrity architecture is a fundamental principle that governs molecular self assembly and how living cells self-organize themselves to respond biochemically to mechanical forces. His work on tensegrity has inspired development of DNA self-assembly-based nanotechnologies, and his insights into mechanobiology have led to the creation of shear-activated nanotherapeutics that selectively target clot-busting drugs to sites of vascular occlusion; he also has developed engineered tissues and cancer therapeutics that have entered human clinical trials. Dr Ingber has authored more than 375 publications and 85 patents, and has received numerous honors including the Holst Medal, Pritzker Award from the Biomedical Engineering Society, Rous-Whipple Award from the American Society for Investigative Pathology, Lifetime Achievement Award from the Society of In Vitro Biology and the Department of Defense Breast Cancer Innovator Award. He also serves on the Board of Directors of the National Space Biomedical Research Institute, and is a member of both the American Institute for Medical and Biological Engineering, and the Institute of Medicine of the National Academies.

Q Your academic career started with a degree in biophysics and biochemistry. How did you transition from this background into cell biology and bioengineering?

I earned both a bachelor's degree and a master's degree in molecular biophysics and biochemistry as a student at Yale University (CT, USA), which actually enabled me to take a broad range of courses in the humanities, art and literature, as well as the sciences. I also studied biology quite extensively, including

developmental biology, cancer biology and molecular genetics. But I was studying in the mid 1970s, just before the cloning revolution hit, which meant it was largely classic bacterial genetics. As an undergraduate, I worked with Paul Howard-Flanders on DNA repair at Yale University, and then I won a fellowship to go to London to do research at the Royal Cancer Hospital in Sutton (UK), where I worked with Ken Harrap, who was working on cancer therapeutics at the time. I worked on the effects of steroid analogs on

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protein phosphorylation in the nucleus, but we were largely taking a cell biology approach, isolating and fractionating cells to look for mechanisms of actions.

Q Your early work led to the discovery of a universal set of building rules for organic molecules. What first prompted you to investigate tensegrity?

When I came back to Yale University, Ken gave me the name of Alan Sartorelli as someone I may consider working with. He was the Chairman of Pharmacology at Yale University at the time, and later head of the Yale Cancer Center (CT, USA). Sartorelli told me about the projects I could be working on: one that excited me was on cancer metastasis, and on the role of glycosaminoglycans in this process. This is where I first learned how to culture cancer cells and where I first watched them stick and spread on a dish. One of the defining moments in my life was approximately a week after I started in the cancer laboratory, when I was taking an undergraduate sculpture class. It may seem strange that as a molecular biophysics/biochemistry undergraduate student I was taking an art class, but I've always been a visual person. I had seen people walking around campus with sculptures that looked like geodesic viruses and so I asked them what course they were studying. They told me it was a course called 'Three-Dimensional Design', and it was a sculpture class. I had tried to get into art classes at Yale University, but it was virtually impossible because there were so many students interested and so few classes. I had given up on it; however, I had a girlfriend at the time who majored in art, and she was a sculptress. The teacher who taught the 3D design class, a man named Erwin Hauer, also taught a human figure class that she was taking. She introduced me to him, and he asked why a scientist would want to take his class. I replied that everything with biology is essentially based on 3D design at the molecular level, such as the DNA helix or actomyosin filament sliding; it's the shape that governs their function – and I got in the class!

The first task we were given was to build structures out of sticks and strings, but the sticks could not touch each other; they had to start lifting off the ground as solid structures as a result of being connected by strings. It was a 3-h-long class, and at the beginning nobody had any idea what to do. I think some of the art students must have seen tensegrity sculptures by Kenneth Snelson before and someone eventually worked out how to do it. At the end of the class, Hauer came in and he had a round structure made out of sticks and elastic strings, and as he was talking, he would push it down flat, and then he would let go and it would bounce up in the air. It looked just like the spread cancer cells I was

culturing when we clipped their anchors using trypsin and they rounded up and popped off the dish. So I just assumed cells must be built this way.

At this time, around 1976, the first papers were coming out about actin and the cytoskeleton, and I was working on a drug called ICF159 in the laboratory that appeared to cause cancer cells to change their shape as part of their action. One day in the laboratory after the sculpture class when we were testing the drug, the cells became extremely flattened and I remember saying to the postdoctoral scholar that "their tensegrity must have changed". He asked me what I meant and I explained that I was taking a sculpture class on tensegrity structures and Buckminster Fuller (who coined the name 'tensegrity'), and he just cut me off! So I kept quiet and went to the library and I started reading. The more I read, the more I started to see that tensegrity could explain things that could not be explained in biology. I also took a developmental biology course around this time in which we watched videos of cells moving and of embryos developing. These films revealed to me that development is an incredibly physical process, and when I went back to read the early developmental morphologists, they also described virtually all of development in largely mechanical terms. Soon it became clear to me that tensegrity could provide a way to explain how mechanics can influence cell structure and function, and guide development. Soon after I started my MD PhD program at Yale University, I eventually shifted my focus slightly and moved to the laboratory of Jim Jamieson in the Department of Cell Biology where I could study both embryological development and cancer. That is how I really got into cell biology and began to pursue the tensegrity area in the laboratory.

Serendipity is an absolutely critical part of science, and certainly, of my life. If I did not have that girlfriend at that time, I would never have been able to take that course and would never have started to think about tensegrity. It is just wild.

Q One of your major research focuses is angiogenesis treatment. What led you to initially work in this area?

When I was finishing my MD PhD and I was thinking about what I would do after Yale University, I heard that there was a combined research/residency in Boston, but that you needed an advisor to apply. The one person I could think of as a potential advisor was Judah Folkman. Judah had worked on angiogenesis and cancer for many years and everybody knew about his pioneering work in those areas. Most people did not know, however, that in 1978, he had published a major article in the journal *Nature* that suggested that a cell's

shape could regulate its growth. Folkman had actually come to Yale approximately a year earlier to give a lecture, which I learned about at the last minute and attended instead of showing up at another meeting I had scheduled. At the end of his talk, I approached him and handed him a copy of a book chapter on tensegrity and cancer that I originally had intended for the person I was originally supposed to meet with at that time, but I never got a chance to speak to Folkman about what I do or my interests. About a year later, I was finishing my clinical training and Jim Jamieson asked what I wanted to do for my residency. I said a mixed residency at Harvard University would be really good, but the only person I could think of there was Judah Folkman. He smiled and explained that he had recently received a letter from Folkman thanking him for the book chapter on tensegrity and cancer, which had actually confused him. I wrote a letter to Folkman afterwards and he immediately wrote back and I went to visit him. That is how I started to work with him; it was not angiogenesis so much as cell shape.

When I visited Folkman, I learned he had discovered that heparin and cortisone inhibited angiogenesis and caused tumors to regress, but he had no idea how it worked. My PhD thesis had revealed that the mechanical properties of the extracellular matrix (ECM) to which cells anchor to in our bodies regulate cell shape and function during tissue development by altering the balance of forces in the tensegrity-stabilized cytoskeleton. Thus, I thought that Folkman's heparin–cortisone combination might work by altering the structure of the capillary ECM. I went to work with him on that, and eventually showed that this was indeed correct, and that we could also inhibit angiogenesis using drugs that specifically target ECM remodeling. We also later confirmed that the ECM regulates angiogenesis and endothelial growth by modulating cell shape and that integrins mediate this mechanotransduction process.

Yet another serendipitous thing happened at this time: one day I had a fungal contaminant in my culture dish. Usually this causes the capillary cells to pop off the dish and die, this time when I looked under the microscope, I saw that the fungus did not kill all the cells, it actually induced a gradient of cell rounding. I thought that perhaps the fungus is secreting something that regulates capillary cell shape, which I had just shown regulates cell growth, and hence, that that may secrete an angiogenesis inhibitor. So I decided to culture the fungus, but I did not tell anyone at first because I was overwhelmed with projects as a postdoctoral fellow and I did not want yet another project. However, eventually, I showed Folkman the fungus, and together we cultured it and identified it as *Aspergillus fumigatus fresenius*. When we tested it in

the standard angiogenesis assays, we confirmed that the product it secreted potently inhibited angiogenesis in various models. A company called Takeda Chemical Industries (now Takeda Pharmaceuticals, Osaka, Japan) thought it was very exciting and funded the project. They identified the specific active molecule as fumagillin, made more than 300 analogs and we both tested them in various model systems. We described the final compound known as TNP-470 (originally called AGM-1470) in a publication in *Nature* in 1990 [1], and it remains the most generic angiogenesis inhibitor ever found. It was incredibly potent, inhibited virtually any type of growth factor tested and toxicity was low. It worked in small and large animals and so it was moved to humans. In the Phase I trial, there were a couple of complete remissions. Some of the clinicians leading the trials were trying to move the drug to the next phase, but for reasons that are still not clear, at this point TAP Pharmaceuticals (IL, USA; a Takeda Pharmaceuticals partner that was leading the trials in the USA) shut down the trial. The media often describes that the trials were stopped owing to neurotoxicity, but that was not case.

Q Do you think nanotechnology could have a role to play here?

In fact, owing to general concerns of neurotoxicity, Folkman and Robert D'Amato developed a nanoparticle formulation of TNP-470 called capillostatin that provides the same antiangiogenic activity, while reducing neurotoxicity, and it also can be given orally. But in a more general sense, vascular biology is likely where nanomedicine will have its greatest impact, and it is already beginning to happen. The challenge for most nanotherapeutics is not only injecting nanoparticles, which is relatively easy, but getting the particles through the circulation, across the endothelium, through the tissue and to the right site in the body. I believe that vascular nanomedicine will be the first major success area because injected particles do not have to cross the endothelium or move through dense tissues to exert their actions. It is already happening with imaging agents and the beginnings of targeting moieties and therapeutics. Theranostic nanoparticles could also provide a powerful way to inhibit angiogenesis or other vascular disease, while simultaneously imaging the response to therapy.

Q Your recent publications in nanomedicine have been on a variety of topics, including cancer therapy and thrombosis treatment. Could you update us on the areas your group is currently studying?

Recently, we have begun to harness our knowledge of mechanics and biology to develop new types of

nanotherapeutics. One idea we had was to target the major killers of man, such as atherosclerosis, myocardial infarction, pulmonary embolism and stroke. They all share a common feature, which is that they result from a vascular occlusion, a narrowing of the blood vessel, often from a blood clot. There are great clot-busting drugs out there, such as tissue plasminogen activator (tPA), but only a tiny percentage of patients are eligible to get them owing to the potential of bleeding side effects. Our idea was to use a nanotechnology approach to selectively target and concentrate tPA at clot sites. We started to look at platelets because they target to a narrowing of the blood vessels, and they do this because they are activated by fluid shear stresses that are selectively elevated in these narrowed regions. Inspired by this natural mechanism, we developed a shear-activated drug-delivery system. We used nanoparticles, approximately 180 nm in diameter, that are composed of US FDA-approved biodegradable materials, and we made microscale aggregates of them that are the same size as platelets. We tuned them so if they reach a shear stress that is significantly above normal, they break up into the small nanoparticles, which settle out locally at these sites. In addition, if they are coated with tPA, the released nanoparticles will bind to fibrin clots and remain bound to them until the clots dissolve. When we coated the microaggregates with tPA and injected them into the veins of mice that had experimental pulmonary embolism, we were able to save 85% of the animals' lives, but using only 1/100th the dose of tPA that normally must be administered in solution to produce a similar effect [2]. We are now in the process of commercializing this technology. It is a great example of how harnessing insights from biology and combining them with the power of nanotechnology, you can truly have a unique impact.

We have also been exploring the idea that we might be able to reverse cancer rather than kill it by changing the physical and chemical microenvironment of the tumor ECM. We established a mouse transgenic breast cancer model in our laboratory that is driven by a constitutively active oncogene, which is very potent, and all of the animals consistently get breast cancer over the same time course. At approximately 8 weeks old, the mammary glands of the mice are normal, at 12 weeks they are premalignant, at 16 weeks you see small tumors and at 20 weeks you see huge tumors and metastases – 100% of animals follow this pattern. We used this model to start exploring how we can develop differentiation therapies for solid tumors that prevent cancer progression by suppressing growth and inducing differentiation. We collaborated with James Collins who has developed a powerful gene network reverse engineering approach, and we identified genes,

in particular *HoxA1*, which we predicted might drive cancer progression. When we inhibited its expression *in vitro* using siRNA, it caused the breast cancer cells to stop growing and differentiating quite effectively, but then we had to confront the challenge of delivering this *in vivo*, and this again brought us back to nanotechnology. We worked with Michael Goldberg, who has developed a lipidoid nanoparticle that can deliver siRNA and provide stable gene suppression for weeks *in vivo*. Finally, we also wanted to minimize systemic toxicity. Therefore, we wondered if we could deliver the drug right to the mammary duct epithelial cells that are having the problems, rather than administering it systemically. Recently, we showed that we can deliver the *HoxA1* siRNA-coated nanoparticles directly into the mammary duct by injecting them through the nipple in transgenic mice. Injection of this nanotherapeutic prevented progression to cancer in 75% of animals [3], which is really mind-boggling given that all of the cells still express the active oncogene at high levels. We hope in the future to leverage this type of approach to develop nanomaterials that you might sprinkle onto a primary tumor site after a surgical excision to induce any remaining cells to differentiate and prevent the tumor from regrowing. We are also exploring injectable nanoparticles that could travel to distant metastatic sites to prevent a dormant tumor from becoming cancerous.

Q Another of your areas of interest is the development of 'organ-on-a-chip' systems. Could you explain to us the rationale behind this work?

We have a major effort in Biomimetic Microsystems, or what have come to be known as human 'organs-on-chips'. We use microfabrication techniques to make microfluidic devices with hollow channels that we line with living human cells. We have made a lung-on-a-chip with human lung epithelial cells on top of a porous membrane coated with ECM, and human lung capillary cells on the bottom of the same membrane, which is stretched across the microfluidic channel. On the top of the lung cells, we have air, and we flow medium over the tops of the capillary cells, so we have essentially rebuilt the alveolar–capillary interface of the lung air sac. In addition, the walls of the device are flexible, and we apply cyclic suction to side chambers that cause the tissue–tissue interface to rhythmically stretch and relax, hence faithfully mimicking the breathing motions of the lung. We demonstrated that using this lung-on-a-chip, we can measure drug efficacies, detect toxicities and model human diseases, such as pulmonary edema [4,5]. We also used these devices to study nanotoxicology in the lung. For example, we showed that nanoparticle simulants of airborne particulates

only induce injury and inflammation in the lung-on-a-chip when exposed to physiological breathing motions. We also discovered that breathing increases the absorption of nanoparticles from the air space into the vascular channel by almost tenfold, and that these mechanical motions are critical for induction of pulmonary edema by the cancer drug IL-2. Moreover, we confirmed these predictions in animal models. We are now developing more than ten different human organ chips, and linking them to together to form a human 'body-on-a-chip' with the goal of replacing animal models over time for drug development, and testing of chemicals and cosmetics, among others.

Q You are the Founding Director of Wyss Institute for Biologically Inspired Engineering at Harvard University. What are the main research projects currently underway at the Institute?

The institute is extremely broad, with both medical and nonmedical projects ongoing. It was based on the idea that engineering has transformed medicine over the past 50 years, but we feel that we have learned so much from biology that it is now time to leverage biological principles to develop new engineering innovations, particularly in the areas of materials, devices and nanotechnologies. One thing that we really focus on is translation; getting fundamental discoveries out and into the clinic and into the market place. We have business development people and entrepreneurs-in-residence working side-by-side with students, fellows, staff and faculty. We focus on intellectual property generation and protection, and we employ over 40 expert scientists and engineers who bring extensive industrial experience in product development into our effort. It is a new model for academia.

Looking at some specific projects, David Mooney leads a platform focused on programmable nanomaterials. The lead project is a cancer vaccine using a nanofibrous polymer scaffold. They have found their approach causes complete regression in approximately 50–90% of tumors in animal models. The results were so exciting in melanoma that they quickly obtained FDA approval to start a human clinical trial at the Dana Farber Cancer Institute (MA, USA). George Church has a platform in synthetic biology, and along with James Collins and Pam Silver, they are engineering microbes and developing cheaper and faster DNA sequencing and DNA synthesis approaches. William Shih, Peng Yin and George Church are also doing work with programmable DNA-based nanomaterials, using DNA origami-based self-assembly approaches, which has also attracted great attention recently. Interestingly, William and I collaborated to fabricate a circular DNA molecule that when combined with DNA 'staples' self-assembles into a 3D prestressed tensegrity structure,

just like one of the stick-and-string models I built in my undergraduate sculpture class, but on the 100-nm scale.

Q Of your more than 375 publications, if you had to choose one as your favorite, which would it be and why?

My favorite publication is the one that people have never read: it is a theoretical book chapter entitled, "Cells as tensegrity structures: architectural regulation of histodifferentiation by physical forces transduced over basement membrane", which I published in 1985 [6] that essentially lays out all of my strange ideas relating to tensegrity, mechanobiology, development and cancer that I pursued for the rest of my career, and eventually confirmed experimentally. It was also literally the last chapter of my PhD dissertation. In terms of high-impact papers, my *Science* paper in 1993 showing that integrins mediate mechanotransduction, and also that cells behave like tensegrity structures, is very important to me [7]. I published another article in *Science* with George Whitesides in 1994 called "Engineering cell shape and function" [8] that was the first to use computer micromanufacturing methods combined with a nanotechnology-based molecular self assembled monolayer technique to fabricate culture substrates that control cell function by dictating their shape and position on the micrometer scale. It was a very high-impact paper with thousands of citations, but not as high impact as a later paper that we also published in *Science* in 1997 called, "Geometric control of cell life and death". In this article, we unequivocally showed that cell shape distortion is a key regulator of cell fate, and that cells can be switched between growth and apoptosis by altering cell spreading independently of ECM adhesion or growth factors. This paper has now been cited approximately 3000 times [9].

Q Finally, if you did not work in science, what would you do?

That is a hard one! I have done some film work and writing in the past; and I love to write, so it be writing in some way. It would probably be something such as a writer/director for television, film or some other visual communications medium. It combines the visual aspect with writing, communication, art and humor.

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