>> Welcome to the podcast series of Raising the Bar Sydney. Raising the Bar in 2019 saw 21 University of Sydney academics take their research out of the lecture theatre and into bars across Sydney, all on one night. In this podcast you'll hear Hala Zreiqat's talk 3D Printing Our Way to Better Health. Enjoy the talk.

[Applause]

>> Thank you, Sarah, for this nice introduction. And thank you all for being here this evening. I thought I'd be by myself talking to the marketing people, but this is wonderful. I know you're all waiting to hear about the 3D printing. You need to wait a little bit more because I do have to give you a context before we get to the 3D printing. So I'd like to start with giving you a story that touched me and I'm sure it's going to touch you. Last year I was at Harvard University as a fellow at the Radcliffe Institute for Advanced Study where Harvard undergraduate students will apply to undertake a project with me. In this case it was on 3D printing, considered to be a sexy subject. So of course I had quite a number of applications. The CV of one applicant particularly interested me. So I asked her to come into my office for an interview. What I didn't expect was to see this lovely young lady walking into my office on crutches. Lin had a disease called osteosarcoma which is a cancer of the bone that resulted in her taking an operation to remove the cancer but she was on crutches because of the consequences of that operation. So Lin was diagnosed at the age of ten when her family moved from Vietnam to Singapore so that they give her the best medical treatment possible. Which resulted in a series of chemotherapy and then an operation to remove that part of the bone that had cancer and replace it with a metal implant. Something like this. I'm holding here a metal implant and I'm happy to have this passed around. It's not the exact shape, but this is a metal implant that is currently used in patients in orthopaedic surgery. The implant lasted one year and then it had to be removed and another operation had to be done simply because the implant did not bond to the bone around it. Her next operation failed exactly for the very same reason. We didn't have enough bone, so we needed to replace the metal, have another metal and then look for another bone to fill in that defect. Imagine being ten years old and each year you have to have an operation to replace the metal, to find a bone from somewhere in your body to replace that metal. And why is that? Because we still to this day do not have available treatment that can be used to treat patients like Lin whereby we have enough bone to fill in the defects. We simply do not have enough spare parts or enough spare bone in our body to keep using it, to fill in the defect or the ever expanding defect in her case. Fast forward eight years, Lin had ten operations to this day. I keep mentoring Lin. I talk to her all the time and we're still worried about this operation. So we are desperate. The issue here is there is no available material that can help people like Lin and many, many other people. Being with a young lady like that and witnessing this is a constant reminder of the clinical unmet need that we face in this field, in the field of orthopaedics. One of the biggest challenges in the orthopaedic space is the fact that we cannot to this day regenerate or rebuilt

large bone defects, particularly when they are under nodes. And this happens – you could get a large bone defect if you have an accident. You lose parts of your bone. You could get a large bone defect if you have a cancer-induced bone loss like we saw in the case of Lin. Or any other diseases or trauma can result in defects like that. In these situations, as we saw in the case of Lin, we have to rely on the metal. The metal has to be placed with a large defect of the bone and try to stabilise that defect. But where is that piece of bone going to fill in that defect? It has to come from either the other side of our body or from a donor. A donor being other human – that human of course. Or being from other species. And that has not been really that successful. We are desperate to find a new material. Lin is not alone. The problem with musculoskeletal diseases affect a huge number of people around the world. And the figure actually states how significant the problem is. In the US, the figure spent on treating musculoskeletal diseases in 2016 was $40.5 billion. When we looked at bone grafts, bone graft material, that was around $1 billion and I only have the figure for the US. $1 billion spent each year and that's growing 13% each year. Now this is a problem. And people have been trying to find solution for this. People have been trying to find a material that can be placed in our body in a large bone defect that can regenerate bone, that can resemble the architecture of the structure of the bone, that can withstand the load. We have failed. We still have not found that material that can be used in the clinic. There is not such a material. It is not enough to simply stay with the 20th century technology. We need to constantly look at the materials that we can use to replace that 20th century technology. And in that I mean we need to develop a material that has the same architecture and the structure of the bone but also biologically encourages our bone once placed in a defect to regenerate and to regrow. Wouldn't it be ideal if you had a large bone defect, let's say in your hand or in your legs or anywhere else, that you go to the doctor and the doctor says, "Oh, perfect. I have a certain material that we'll just place it in that defect and you'll get brilliant bone forming and no problem if there is load applied to it. It will still withstand the load." Isn't that what we're striving to do? It is. And this is what everybody in the field is looking into, and this is why 13 years ago I moved from the University of New South Wales where I was in the faculty of medicine and moved to the University of Sydney in the faculty of engineering, to do exactly that. The aim of the lab was, can we develop a material that if we place it in a bone defect can regenerate bone? Can continue to send signals to the surrounding tissue in that defect and ask it to make more and more bone, but at the same time be mechanically strong and support the defect? People have tried to do that. So far we have materials available but the problem with that most always, almost always we have to add to them some biology. We have to add to them some cells, some growth factors to induce the bioactivity of the materials. And that comes with all sorts of hurdles, of regulatory body approval issues, expenses of course. It's very expensive. And safety issues. The other thing with the mechanical – I talked about the mechanical. The mechanical problem was difficult, still difficult to this day to accomplish. Because if we have a material that's mechanically strong – now how many of you know how

the bone structure looks like? Bone has inside it like a spongy-like structure, what we call Swiss cheese if you like. And surrounded by also we have solid bone. And that's therefore the reason these two structures provide the stiffness and strength to the bone. If we want to develop a material that's highly porous, you're automatically compromising the strength. The mechanical strength and bioactivity are always interlaced together. So if you change – if you increase the mechanical strength, you always have to reduce the porosity. And if you reduce these pores and holes, this means you don't have enough bioactivity. We don't have enough cells and fluid and blood vessels to go throughout the bone. For many, many years we thought the bone is a dead tissue. Not until we were able to get these technologies that allows us in the 80's, that allows us to cut through the bone and look at the structure of the bone. So that's why this method has been really, really difficult to accomplish. And before I go to what we are doing now which is the 3D printing, which all of you are sitting here waiting fort that – you do still have to wait. Because I think we need to go back to history. Doctors and – I mean, I was always fascinated by the creative way humans have relied on materials available to them to use in surgery or implantation. And this goes back many, many years in history. Back 7,000 years ago, people have used forever materials that they can find available to them to fix bones. An example, 7,000 years ago a skull was found in Piro where there was a gold plate that as used to fix the fracture. Almost at that same time, ancient China used bamboo peg to replace teeth. So that has been happening for a number of years. In 600 AD the Mayans very beautifully used the shell of the mother of pearl, nacre, to replace teeth. And what they found, why did they use nacre? Nacre is tough, it's strong material. But they demonstrated very beautifully that it actually can regenerate and build the jaw. So they were very clever. Centuries later in 1668, a Dutch doctor by the name of Jacob Macaran – he was one of the doctors in a war zone. And a soldier was injured in the skull. How's he going to fix that skull? So they turn to a dog skull, took a piece, exact matched piece of the side to fix the skull of that soldier. That was the birth of bone grafting then. And it healed so beautifully. Then the soldier was excommunicated from the church because he was labelled part dog. So he went back to the doctor and he said, "I don't want this. Please remove that." They could not remove it. It had healed so beautifully. Now despite the really significant need and clinical unmet need of materials like that to fix bone, they stopped using that now for the next 150 years or 155 years. We don't know, is it because of religious reasons? Is it because of cultural reasons? Nobody knows that. But then more than 150 years later, Dr. Walton from Germany – they needed to do something. So they said, "Okay, we can fix missing pieces of bone by taking another piece of bone from people from the same species. Human to human, dog to dog, cat to cat." And that solved the problem. And that was the first birth of bone graft substitute now. Autograft we call it, material taken from the same species.

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>> So the history of implant really all relies on an ad hoc basis. Doctors look in their backyard and so what is it that they can use to treat certain diseases

or defects in this case? And if successful, that was by pure luck. There was absolutely no rational designs, no strategic rational experimentation that led to this. And we fast forward now to the 20th century. 20th century was the first birth of rational design and experimentation to develop materials that we can use in our body. Now I know many of you know of a hip replacement, right? But we don't know the history of that. 1932, a doctor called Smith-Peterson in Boston I think – he was the first to use glass to regenerate or to fix cartilage which is the layer that covers the bowl of the hip to fix that cartilage. Can anybody tell me why didn't we continue to use glass in this area to fix cartilage? Because weight is applied to it, so there is stress applied while we are walking, right? And that stress will just shatter the glass. So we cannot use glass in a load-bearing application. It just shatters. But we still need the material. So they continued to look for materials and looked for what's available in the industry. Vitalium, it's a metal allow we call it, so it has cobalt, chromium and molybdenum. And the doctor called Dr. Venable – so he used that metal, put it in the body and that's perfect because it was electrically inert so it didn't introduce any electrical field once in the biological fluid or in the body. It did not corrode and it did not induce any foreign body reaction. So here it is. We've used now vitalium. And this is the material still used in this day for screws and fixing minor fractures. Now we still need to come up with a material that can fix hip, total hip. So in 1940, another brilliant doctor by the name of Austin Moore, he then made a whole hip – so a whole leg and a bowl of the vitalium to place a bolt into the shaft of the femur. And that then becomes the total hip implant. And then he modified that to become the modified Moore's implants. Not until 1960 that what you guys know now of the hip replacement has come into play. And that's due to a famous and most clever British orthopaedic surgeon by the name of Sir John Charnley who worked very closely with the engineers to come up with a design, a three-piece hip that can be used in total hip replacement. And this is the most successful operation and the landmark of the 20th century total hip replacement is this implant which is in use to this day today. So we owe much of the modern hip replacement to Sir John Charnley. Now orthopaedic implants of course would last for around 8-15 years. 20 years ago, no problem. Because they would be put in a patient of 70 or 80 years old. But now we live in an age where we have ageing population. The younger population are very active, so constantly wear and tear exposed to the hip. So really we just cannot be satisfied with what we have right now. We need to do better. And they will eventually lose? How do they lose? You then have a fibrous tissue that separates the bone from the implant and we need to change it. So what are we – and I'll give an example. You don't even have to be old. A cousin of mine at the age of 35, she had – she needed a total hip replacement. Why is that? Because when she was 19 years old, she had an autoimmune disease, systematic lupus erythematosus for which she had to take a drug, prednisone, which then poisoned her bone. Now she's 50-something years old, so how many more implants will she need? So nobody is immune. So you don't even have to have rheumatoid arthritis or osteoarthritis or any disease of the bone that leads to this. So what are we

doing and what others are doing? We're all working in the field to develop the ideal material that can have the biological and mechanical properties suitable for building new bone. The two crucial prerequisites, as I said, is mechanical and biological. And I talked to you earlier about why we cannot have a material – why we have not yet produced a material that has both of these properties combined together. The holy grail in my opinion in a material that can be used for this is one that can satisfy both the mechanical properties matched with the biological properties into one material. And this is exactly what we set out to do some years ago. And to that we have returned to the glass, just like that, just like this cup of glass that I'm holding. We wanted to turn that glass into the material that can then be used to build bone. Or ceramic. So it's a ceramic, not glass, sorry. Ceramic. Pretend I showed you a cup of ceramic. So to achieve this we looked at the property of the tissue that we want to help rebuild. In this case it's bone. The bone has calcium. Bone has trace elements like strontium and zinc that are all important in bone formation. We thought, "Okay, how about if we mix all these elements together and come up with a new material that maybe perhaps we will be lucky to have a material" – I mean, this was a rational strategic design, but also we need luck in any research. Maybe that will lead us to the material that we wanted. So we have incorporate strontium and zinc in different concentrations and now we have developed a material that can regenerate bone. And I have this material here. I have it here. What I'm showing here for the podcast is ceramic material that is now 3D printed and has holes in it. 70% porosity. 100% interconnectivity. And I'll pass it around. If you feel it to see how strong it is at this very high porosity. And if we made that, that's 3D printed. If we made that material less porosity it will be even much, much stronger. And what we did, we took a piece of bone from the tibia of the shape. Now that is large enough that it would not heal on its own. Help is needed. And here we put this material in the shape. No cells. No growth factor, nothing. It's just sterilised, press fit in the defect. It has to match the exact defect it's going to go into. Here this material is printed with that structure, put in the defect. We left it for three months and one year and we found by one year the whole defect was filled with new bone and wherever there is new bone with the appropriate properties has formed, the material started to degrade and disappear. Yeah. It's beautiful. And this is the first – yeah.

[Applause]

This is the first in the world. What we did then, all our work is about translating and taking the product to the patients because of many things. First of all, it's extremely hard work, long hours working in the lab to develop something like that. Second, we rely on funding from the government, National Health and Medical Research Council and Australian Research Council, and that's your money, taxpayers' money. So we need to give you something back. And that's one of the – this talk is giving you back as well. So we need to do that. And it is very, very important, every search we do, that we translate it to the clinic. We work very closely with a company and I'm proud to say it's an Australian company who make this product, so it stays in Australia. It's global. They

signed global licencing agreement for it. Now we need to work very closely with them to design the product such that it gives us the utmost bone formation when it's placed in vivo. And what we have found again with the brilliant post-doc that we have in the lab, that I have in the lab, a pure ceramic guy who managed to now print this material not only with that shape – which we were limited at that time with this shape – but to print it in complex structure now. And the idea is we want to see if we change just the architecture and the structure of the bone, can we actually affect the amount of bone that's formed? And we just found that – a paper just got published a few months back where we found that we work with the same composition with the material – we haven't changed chemistry. We haven't changed anything. All we changed is the architecture and the structure. We can significantly affect not only the amount of bone that's formed but also the quality of the bone. So you could turn the material from forming sick bone to healthy bone just by changing the architecture. So we work very closely now with a design of the material to take it to the patient, and that's by virtue of the 3D printing technology that allows us to do that. Because without that, we cannot go into this. Another thing that's really important, if you look at bones – so we have developed this material. We know we have bone formation, all of that. But if you look at the structure of the bone again, another important aspect that you need to know, it's a hierarchical structure. It starts from a nano-sized level up, up, up to a macro-size level. Bone has this hierarchical structure but also has, like you all know, something called mineral which is calcium phosphate which is like chalk. And also has a material like that protein in our skin, collagen, soft material. Now the strength and the toughness of the bone is not because we have that chalk and the collagen. It's because of that hierarchical structure combined together gives this bone a specific toughness and strength. So if we cannot make a material like that, we are not going to produce that tough and strong material like that of the bone. So the next challenge for us which I'm very glad to say that we're almost there – and again by virtue of the brilliant post-doctors I have in the lab. We're now trying to develop the technology of the 3D printing that goes down to the nano level, print bone in the nano hierarchical structure that we have. Again, ask the material – the big question is, go and build bone, wherever you are. And the next thing we are doing – so the first application in the human – sorry, I forgot to show that. This is for spine fusion. And the printed material here that I'm showing which is a spine case, this is the first application that's going to go into humans hopefully in the very near future. I'm not going to put a timeline on this. And again, you can pass that. So the most – so we are using really sophisticated technology, the most sophisticated 3D printing technology known to man to be able to produce that at the nano level. And this is not minor. Where we're going next now – and we started this work almost last year – try to print a whole tissue in a dish. A whole organ, if you like, in a dish. And this is another brilliant guy in the lab who actually built the 3D printer. All of this done by 3D printers built in the lab by the people I have. We just buy the machine. $3,000 is very cheap in the scientific terms. So bioprinters cost around $85,000. I'm talking $3,000. But these brilliant people try to adapt

this 3D printing technology to enable us to do what we can do. So we are now printing a broader application. We want to print tissue in a dish. What we are doing is printing a material in a dish where we can have the material to instruct the cells, stem cells if you like, that we put on the materials with different cues in the material printed, to tell the cells to make certain cell types in different locations. Just like you're developing embryo. And I think we are underway for this. I cannot talk about that right now because we haven't validated our results and our experiments. But we can go around millimetre-size 3D printed organs in a dish, instructed by the materials that we print, by the growth factors or the biological cues we put onto it to hopefully one day make in the near future, make a whole embryo in the dish. That's really important as well. I mean, I started to hear talking to – I give a course at the university called Principles of Tissue Engineering where we talk about organ donation. And this is a big problem around the world. We don't have enough organs available for people who suffer from failed organs. So if science allows scientists and technology allows scientists to print organs in a dish, that will alleviate the problem of many, many people around the world. Maybe one day you can go, "I'm going to buy a liver from .com or something." I don't know.

[Laughter]

What we are also doing here is – I'm sure many of you have heard of people who have cancer-induced bone loss here in the jaw. Many people will lose the jaw, right? And the problem is printing a jaw like this, a whole jaw like that. We are now and we are working very closely with my collaborators and colleagues at the university in the mathematical modelling where they model the defect and look at the stresses that's going to apply on the material in certain areas of the defect. And tell us then when you design your material, you need to make sure that if you design it weak here, that's not going to work. You need to design the material so that the stresses applied to it are going to be factored for when you print the material. And we work very closely with these mathematical modelling people to now model the whole jaw. So hopefully one day the surgeons don't use metals, because that's what they use at the moment. A big chunk of metal put in your jaw. So maybe we can use this and the material will then be remodelled and build new bone and at the end of the day you'll replace the whole thing with bone. I don't know when is that going to happen. But hopefully that will happen. Another dream of mine is that we let go of all of these metals and have the ceramic be your total hip replacements. I'm going to finish with where we're going next, only a few sentences. One of the other projects in the lab is nanomaterials. And this is nanomaterials which is a nano-scale material, 10 nanometers, 14 nanometers-scale, nanoparticles where we are going to use those. People are using those for delivering of cells or drugs to a targeted area. But it has all sorts of problems. What we are trying to develop is magnetic nanoparticles combined with functional nanoparticles as well. Which means they are magnetic by they also have the right cargo that we want to deliver to a patient. So imagine you have a cancer patient and you need to deliver a drug to that site. So I don't know, maybe brain actually because nanoparticles are

very hard to cross the blood-brain barrier. The ones we are developing, we have evidence that they do. And the idea here is you load it with the cargo, you send it straight to the cancer area, you apply a magnetic field. It releases the cargo, it releases heat that will kill the cancer cells, attach it to the nanoparticle, but your normal cells will not die at 42 degrees. That's where we are. So watch this space for promising, fantastic research coming not only from the University of Sydney but from all scientists around the world. And thank you so much for listening.

[Applause]

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