No One Can Truly Measure Exosomes: The Truth About Exosome Quantification Transcript

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Podcast Introduction

Hey there, welcome to the Recharged Biomedical Podcast. I'm Dr. Edward Park, and if you're curious about regenerative medicine, you've come to Hi everybody, this is Dr.

What Exosomes Are and Why Counting Matters

Ed Park. I want to talk about quantifying exosomes and what we know, what don't know and we can speculate. Here is the PowerPoint. It opens with Erasmus' quote saying, in the land of the blind, the one-eyed man is king.

And unfortunately the GIFs don t animate, but here is Steve Carell as Michael. Who is blind and groping at straws here? So I always compare it to gasoline.

You pull into a gas station, you don't wonder if you're buying something other than gas. Above me is the distillation of petroleum products and different layers and processes cause different end products.

Is this what exosomes are like? No, it is not. The question always arises from doctors who've switched brands. Well, why would they lie? They say they have exosomes.

Here's a chart comparing six different brands of so-called exasomes, and the obvious answer is the one we find in most affairs, which is they lied because of money.

If we give people the benefit of the doubt, perhaps they're just ignorant. And so the pie chart above reminds us that What you know is a very small fraction.

What know you don't know, is larger. And the biggest is what you do not even know. I was watching a video by Stella Cormbonis, one of the Harvard researchers that was mentioned in my book.

She was talking about how they just knew how many roughly millions of stem cells they had. In 24 hours, that is the amount of exosomes they would collect.

Then they go through their filtration or centrifugation.

Why Exosome Output Varies by Cell Conditions

So the question arises, how many are these things even making? It's hard to count them individually, or I would say impossible. So we can liken it to chicken husbandry.

You have these chickens in a factory farm and they lay eggs. Apparently, if the infographic is to be believed, ancient chickens used to lay only 12 in nature.

In the early 1900s, they got that up to 120, and nowadays it's up 300. Why? Because they're living things and based on the amount of light they get, calcium, the hygiene, whether they to free range, are they different breeds?

Are they young chickens? These all determine how many eggs they will lay. In similar fashion, when you have mosaical stem cells in these flasks and they're laying their exosomes.

They're musicians, they are live. they will bury their output depending on their environment, which could be something like temperature. Oxygen definitely affects it.

Stress, whatever that means, basically play like punk rock, They put more exasomes out. pH is known to affect it, norepinephrine, adiponectin, and also very crucial number of population doublings.

So how old is the lineage? After about two, three months, they start to senesce by various things such as epigenetic silencing of the genes and telomere shortening.

So you should not be using exosomes from exhausted, older chickens, so to speak. Now, the key part of what we're doing here is getting rid of big stuff.

On the upper left, you see that a cell is 20 to 50,000 nanometers. An exasome is only 100 nanometer. Microvesicles which are sucking in and sucking out of the cell are, you know, anywhere from 300 to 1,000 nanometers.

Apoptotic bodies which have destroyed fragments of cells can be huge, up to 5, 000 nanometer.

Filtering Out Large Particles

So let's look at an analogy above. We got the sand which is the exosomes, the cells which the rocks, and even the microvescicles, which can easily be filtered out.

So at the bottom left, you see here that we have this tangential flow filtration. You flow the solution. Everything that is small enough will fall through the grates like in the street and everything else will get filtered out.

So the rocks. The pebbles filtered out. And if you like sushi, it's more like flying fish eggs or tobiko is above me. Those are tiny eggs. We don't want the big eggs, which are the microvesicles or the ikura.

But we don' want to salmon, of course. So dirty little secret, what does nano mean? Well, there's no strict definition, but what we're talking about is that light can only be visualized at 200 nanometers or above.

These are invisible to us. Now, electron microscopy uses a different technology. It's not actually using light per se, but optical microscopes can only go to 200 nanometers.

So how do we see these? Well, everyone in the business uses something called a nanocyte. It's basically a machine to count the amount of optical scatter.

So what does that mean? Okay. Well, looking to the left, we see two charts, 100 nanometer polystyrene beads, and then 500 nanometers glass beads. And if you run it through the nanosight, you can calibrate how well it's reading.

How Nanoparticle Tracking Works

Now a little concerning is the longer you run it, the lower the concentration is. I'm not sure why that is, but you can see that the shape of the curve goes down.

So a typical readout from an exoskeleton company might show something like this in the middle, that nanosite. It shows that there's a mean, a mode. This is average size of particles.

And how do they know how big the particles are? Well, this guy with the European accent and the pens in his shirt is explaining to us. So above me or next to my head, you've got the flow of the fluid through.

You got a laser beam of 50 micrometers going through there. And the amount of scatter is registered. The laser is scattered by particles. It's focused into a lens and the optical camera reads it.

Based on the number of Brownian motion and some software, they can infer the size and number of the particles. And that formula is shown in the middle.

D is light dispersion equals a constant kb. Temperature influences it, so it has to be at the same temperature. It's over 3 pi V, which is a viscosity coefficient, and D the density.

So this is all software inference of how many and how big the particle are. But unfortunately, it's all in the wrist. What do I mean by that? Meaning if you change the parameters of your counting, the sensitivity, you can take even distilled water and count from zero particles to trillions of particles by changing that sensitivity and by being more inclusive of the area under the curve.

When we look at real electron microscopy, which again is not optical, electron-based, we see that the canonical size of exosomes for various physiologic or geometric reasons is pretty constant at 30 to 150 nanometers.

What Real Exosome Sizes Look Like

I remember talking with one of the founders of an exasome company, showed me his nanosite and it was 300 to 400 nanometer and he said, well, bigger is better, just do the math.

And I thought, okay, that makes sense. But then I looked at the literature and nobody was saying that real exosomes are that big. So he's probably got micro vesicles, maybe even apoptotic bodies in his brew.

How do we know how many exoomes we're buying? Well, unfortunately, we have to rely on Tevye's tradition. It's a tradition, as in Fiddler and the Roof.

In my experience of over five years, the product has always been clear. When we thought that means there's no optically visible particles, it's nano. uh, the potency has been predictable.

I presume that they have the same scientists, same process, and that their doing the thesame thing, unless they're not, which I have to believe. So like Steve Carell says in this, I don't know.

But I do know that a single donor pre-COVID tested for 47 infections or so. They do always have this CD63 and 81 markers, which are MSC exosomes and the BAU or biological activity unit is always high.

One question I've never gotten answered, does anyone use centrifugation for size inclusion?

How Products Are Tested and Compared

It's very easy to exclude big stuff, but if you spin something, you can get the denser, smaller stuff to precipitate below. Does anybody just go for that sweet spot of 30 to 150 nanometers and make sure they're getting pure, pure exosomes?

Maybe not. But I have a strong suspicion based on the next slide that most of the competitors are just using conditioned media. So there are tons of particles in conditioned and we don't really know what they are.

So, let's talk about what we do know. Years ago, I asked the chief scientist of the company I use, how do you know what's in them if you don't break open the exosomes?

He said, aha! We do. So above us, we see Triton X100, which is an industry standard. You can see it's amphiphilic, meaning it has a polar head and a non-polar tail.

This is AKA soap. That's what a good soap is. So you gotta crack open the eggs, you got to open up the toy bubbles to do your proteomics, to your RNA omics.

And that's exactly what this company did. So if you want to go a deeper dive, go to tinyurl.com XOWeb24 as shown, and I interviewed the lead scientists from this company that I've been using.

Here's a mashup they did recently of five competitors. Now it's very concerning. And of course, I have no way of

knowing if this was truly a level playing field.

Did they use the Triton X100 on all their competitors? I don't know. I presume they. But what we see here when we compare side by side is that only three of the products had even the CD81 marker.

That's concerning. When you look at the concentration of exosomes by HPLC, or high throughput liquid chromatography, the industry standard for detection, there was 100 times more exasomes in this product than the others, which had 1.9 and 1,7. Now that's micrograms per milliliter.

But most concerning when you look at the biologically active units, meaning you melt open the contents with the Triton X100, you quantify the RNAs and the proteins, and you do a little format which is described in that lecture to the left.

Closing Remarks and Resources

We have 250 to 900 times the biological active unit in this product versus their competitors. But it's very damning if the results can be repeated. And indeed this company has said that they will cover the expense of comparing a competitor's exosomes in their presence.

Okay. As Steve Carell or Michael said, just be smart. I don't know how much I know. Probably, I, don' know a lot, but it seems to be a good product. If we are to believe their testing results, they're very concerning as to the actual veracity.

One doctor I had come back to, to our brand, not my brand. Now I have a fiduciary relationship with them per se, she had been using and I've met, many doctors have switched out and come back and it's just a better, more predictable result perhaps because the other brands are mainly just condition media.

So we're looking at contacting me. You can scan my barcode. you can download five years worth, the first five of years of educational blogs and videos.

My contact info is there at www.ovu.me slash Edward Park. Sign up for my newsletter at rechargebiomedical.com. My YouTube channel is drprk65. You can subscribe.

Email me at that email and pick up a copy of my audio book. I describe pretty well what exosomes are and how they can help you. So that's it. I hope you tune in to the next video and thanks for watching Dr.

Edward Park on quantifying exosomes. Many clinicians are getting interested in exostome therapy and they hesitate for good reason. Questions like, does this work?

What forms do I need? How much should I charge? how do i stay out of trouble? All these questions are addressed in my online course. That's why I created it to help you get started.

The online course is your permanent turnkey resource to get started either treating yourself, friends and family, or to expand your practice and help more people, as well as increase your revenue.

Thanks for tuning into the Recharge Biomedical Podcast. If today's episode got you thinking, you'll love my book, Exosomes, Songs of Healing. It's packed with cool analogies, full color illustrations, and all the science you need to understand how exosome are changing the game in aging and regenerative medicine.

You can grab it in paperback, ebook, or audiobook, whatever works for you. Now head over to www.rechargebiomechanical.com to check it out. And don't forget to like and subscribe so you never miss another episode.

See you next time.