Nanomedicine literature: the vicious cycle of reproducing the irreproducible

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Current scientific research is facing an unprecedented challenge in the form of an ongoing reproducibility crisis [1]. This is alarming due to the fact that reproducibility of published data remains one of the key requirements in advancing scientific research. In a recent survey conducted by *Nature*, approximately 70% of respondents claimed failures to reproduce data reported by others [2]. The corresponding replication failure rates in chemistry and biology were as high as 90 and 80%, respectively. Doubts based on reproducibility of a significant proportion of published scientific literature continue to be rife and bring bad press by making headlines in various news portals [3]. Unfortunately, the scope for such doubts on reproducibility of data also includes biomedical sciences and nanomedicine. It is difficult to ascertain the level of irreproducibility in published literature in nanomedicine. However, it will be unwise to take it as granted that nanomedicine research is immune to such crisis given its highly inter-disciplinary nature and collaboration with chemistry and biology. As an enabling technology, nanoscience is relatively new and any evaluation on the quality of the published literature in nanomedicine is expected to emerge gradually. However, the growth and attention drawn by nanotechnology and nanotech-inspired materials for biomedical purposes are evident [4]. Materials at nanoscale dimensions (0–100 nm) show unprecedented properties compared to bulk

materials and researchers are keen in utilizing these properties to gain edge in various applied fields including drug delivery and medical imaging. Unfortunately, in spite of the uproar, media publicity and favorable funding situations, nanomedicine has failed to offer little in realistic terms. Most of the principles on how engineered nanoparticles (NPs) will achieve exciting feats within the animal and human body still continue to be described on cartoons and ambitious flow charts with very little actual experimental data to substantiate such claims or expectations.

Defining reproducibility & applying it in nanomedicine

One major obstacle in tackling the issue of irreproducibility in scientific research is in fact to define reproducibility. The situation becomes even more complicated for nanomedicine due to its inherent inter-disciplinary nature where researchers from a variety of backgrounds, including physical chemistry and pharmacy, are engaged. The way reproducibility of experiments is explained and the acceptable margins of error differ highly within disciplines. With the advent of newer technologies, novel experimental platforms within nanomedicine research (e.g., new cell lines, microfluidic platforms, *in silico* tools) are being introduced [5]. Such additions and alterations in experimental platforms further complicate the conditions

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for reproducing data. A general criticism in this regard is that even after recognizing such hurdles, there are no globally accepted standardized protocols set for researchers to follow while performing experiments. For example, the definition of NPs still remains controversial and leaves plenty of room for interpretation. While physical chemists generally stick to the widely popular definition of nanoscale to be 0–100 nm [6], much bigger particles, even up to 500 nm sizes, continue to appear in pharmacy journals as NPs. Similar discrepancies and controversial methodological practices are prevalent also in characterization of NPs. For example, questions have been raised with regard to size measurement of NPs based on certain techniques such as dynamic light scattering (DLS) and how it influences cellular experiments [7]. While DLS is known to measure hydrodynamic radius of solvated and diffusing NPs in dispersion, incorrect representations of the light scattering data (e.g., z-average size for poly-disperse suspensions) continue to be used and published. It is common to come across papers that claim to have synthesized nanoformulations (with particles sometimes >200 nm) based on questionable DLS data. Such claims should at least be substantiated with size-distribution plots (expressed on intensity of scattered light) from DLS or imaging data, for example, electron microscopy or atomic force microscopy. Even if any electron microscopy images are provided, they often show low numbers of NPs (often <3) per field whereas a field view of at least 50–100 NPs is required to substantiate claims of synthesis of NPs and further evaluation on size distribution/shape. Similar methodological challenges exist in performing biological experiments – both *in vitro* and *in vivo*. For example, using cell line models (e.g., human colonic adenocarcinoma derived Caco-2) on transwell inserts with different pore sizes (200 nm–3.0 μ m) is a commonly used *in vitro* technique to assess the transport of NPs [8]. Unfortunately there is no widely accepted consensus on the trans-epithelial electrical resistance values for these monolayers to be appropriate. In literature, a broad window of 250–1000 Ω as total resistance has been used [9,10] as trans-epithelial electrical resistance values for such transport experiments, which makes it difficult to interpret, compare or reproduce the data. Adequate characterization of NPs dispersed in cell culture medium containing proteins – known to produce the characteristic ‘protein corona’ – also remains a challenge [11]. Moreover, reports are now emerging that most of these *in vitro* cell lines (e.g., HeLa, HepG2) are contaminated, which raises fresh doubts on the validity of published literature on these cell lines [12]. Such methodological challenges also exist with *in vivo* models which continue to pose challenges in drug delivery

research. Detailed discussion on the deficiencies of the current *in vivo* models falls outside of the scope of this commentary, although there are excellent papers available for further reading [13,14].

Batch-wise variation in NP-synthesis & effects

Maintaining reproducibility during synthesis at nanoscale is a challenging task. During such synthesis, control with high precision over the reaction is of paramount importance, which unfortunately is quite difficult to attain. Even the slightest of variations in reaction environment (e.g., temperature, pH, ionic strength, impurity/contamination, molar ratio of ingredients) can magnify into a perceivable difference in the final nanoconstruct with disparate properties, for example, drug-release profile. Furthermore, with emergence of advanced nanoformulations with diverse molecules (e.g., peptides like insulin, macromolecules) often requiring complicated synthetic techniques including encapsulation, use of biodegradable materials and surface functionalization (e.g., PEGylation), there is no stringent guideline on what should be an acceptable margin of error (e.g., PDI/polydispersity index). Roughly 10–15% variation in size is quite commonly encountered with synthesis of NPs around 100 nm, which is difficult to eliminate [15]. Moreover, a significant proportion of popular tools to characterize NPs (e.g., DLS) becomes ineffective at therapeutically relevant higher concentrations. Therefore, it is difficult to characterize an NP-dispersion with high concentration that is actually being tested *in vivo*. The current state-of-the-art practice is usually to characterize a comparatively dilute NP-dispersion (e.g., 50–100 μ g/ml) and then expect that same behavior to continue at higher concentrations [16]. Unfortunately, this is far from reality and NPs, due to highly reactive surfaces, invariably agglomerate at higher concentrations unless surfactants are used. To a large extent it is impossible to predict the agglomeration patterns of NPs over longer time spells at high concentrations and even equivolume aliquots from same stock dispersion may show incomparable agglomeration. Such unpredictable agglomeration also results in unpredictable behavior, for example, dissolution, release profile and precipitation [17]. This adds further to the batch-wise variation in results. In the large collaborative research consortium between EU countries and Israel called QNano (Quality Nano), this batch-wise variation while synthesizing NPs has been identified as one of the major challenges [18]. Still, many papers in NP-synthesis continue to emerge without any mention of error occurring during synthesis or any statistical analysis of the raw synthetic data being mentioned.

Perspective

One can reproduce/replicate something only when it is known precisely what is to be reproduced. At this stage the knowledge on most of the nanoparticulate drug formulations is far from adequate and the old question ‘*What’s in the bottle?*’ continues to frustrate researchers. It is even more worrying that, despite all these hurdles having been known for a long time, the primary research focus continues to be on developing new formulations instead of tackling the synthetic, analytical and experimental challenges that already exist and inhibit translation. There is already a sufficiently high number of NPs present for drug delivery purposes and we therefore need to prioritize overcoming these challenges instead of producing even more NPs which neither work *in vivo* nor are immune to batch-wise variation. Upscaling remains a significant challenge and very little progress has been achieved so far. Universities are often not equipped for large-scale production of NPs given the significant biohazard concerns plus infrastructural requirements and industrial participation for upscaling of NP synthesis remains far from adequate. Therefore, significant challenges with process engineering continue to hurt the nanomedicine research.

The landscape of current research especially within academia is metamorphosing fast. Introduction of smaller groups and constricted funding opportunities with goal-oriented and deliverable/output-based research are leaving very little space for reflection, which is indispensable for an intellectually demanding exercise like research. Unfortunately, nowadays scientists are often judged and funded based on metrics like h-index or impact factors of journals in which he/she manages to publish. Therefore, pressure to publish looms on young investigators to keep the lab running. Ironically, nature and her mysteries take time before unravelling to the researchers, which is incompatible with the current ‘publish or perish’ culture. As a result, practices like hurriedly designed protocols, lack of including sufficient repetitions, partial and preferential reporting and insufficient account on used materials and methods are widespread. In a recent opinionated article, eminent scientist and Editor-in-Chief of the prestigious *Journal of Controlled Release*, Prof. Kinam Park (Purdue University, USA), has opined that the current drug-delivery research, including nanomedicine, suffers from ‘Gorilla syndrome’ [19]. In this article Prof. Park delved deeper into the current shortfalls in nanomedicine research while mentioning:

“Instead of self-serving praises to each other, we need to be more critical of each other’s work in a constructive way. We need to cultivate the younger generation of scientists with the ability to see things critically, instead of

just accepting whatever is given to them simply because it was given by their advisors or the authority figures in the field. Our current environment is not suited to cultivate frank discussion and critical yet constructive exchange of opinions. Questioning is not disagreeing or disrespecting. It is an ultimate form of respect, but this has been lost.”

In a similar editorial article co-authored by Paul S Weiss, Editor-in-Chief of another prestigious journal *ACS Nano* published in 2015 [20] stated:

“Many scientific articles, including reviews, are written as if funding agencies were the audience. There is typically great potential, a problem that is solved, but little mention of further problems to be addressed.”

So warning comments are being made and it is now to the nanomedicine research community that must address these issues. Skepticism has increased further from serial bankruptcies of nanobiotechnology companies (e.g., NanoInk Inc. in 2013, Bind Therapeutics Inc. in 2016) which were launched by established researchers and were able to attract significant funding initially. Irreproducibility of published data further adds to the misery. It cannot be denied that this reproducibility crisis poses serious practical and experimental challenges in the advancement of nanomedicine. The only way out is to provide detailed accounts in the experimental protocols while publishing. Fortunately, most of the journals are providing unlimited online space for supporting information that researchers can utilize for providing thorough experimental details. It is also important that the researchers provide the raw data as supporting information as well. Societies such as the American Society for Cell Biology (ASCB) [21] and the Federation of American Societies for Experimental Biology (FASEB) [22] have delivered new guidelines to ensure basic standards while doing biological experiments that should be strictly adhered to.

The author has always been in awe of late Prof. Frederick Sanger (1918–2013) – the winner of two Nobel prizes in chemistry (1958 and 1980). In his entire scientific career Prof. Sanger published around 30 papers in mostly journals that were often not the highest rated as far as impact factors are concerned. This also means that his h-index is relatively average when compared with the profiles of most current senior faculty members. However, there is one difference and that is no one could ever raise doubts on the reproducibility of his data. It is amazing how meticulously he kept records of every single experiment he did in his laboratory notebooks. It is of no surprise that his experiments were reproducible because all the methods and experimental details were accurately penned down. It is great news that the lab notebooks of Sanger are digitized now and are freely available online for viewing and download from the Wellcome Library online portal [23]. Such

basic, but highly efficient, laboratory practices are becoming even more important within the premises of nanomedicine, which is evolving fast. It is also important to critically analyze and reflect on the data before inferring conclusions. The Netherlands Organisation for Scientific Research this year has allocated €3M on a replication project that will fund ventures that target to replicate already reported research of high impact [24]. The funding on such reproducibility drive is expected to increase gradually although it will be interesting to see how other stakeholders including sections of media react to it.

Today, more than ever, research including nanomedicine needs scientists like Prof. Sanger who prioritized research before everything else and excelled in it. Prof. Sanger once famously said, “*I was just a chap who messed about in his lab*”. This is perhaps even a bigger lesson for current researchers in academia often busy in multitasking to become eloquent presenters, capable of writing attractive grant proposals and joining the race to become successful entrepreneurs. On the other hand, current scientific literature is in crisis due to the high influx of untrustworthy data and a great deal of taxpayers’ money is continually spent to reproduce experimental data that cost a lot to generate in the first place. It is impossible to find a rational link between these two scenarios. The time has arrived when researchers should be judged and rewarded based on what they have actually delivered in form of reliable plus reproducible data and not how much funding he/she could attract. The myth of more money equals better research also needs to be busted. Finally, the obsession to fund research with

‘impact’ should cease. Impact of research is difficult to ascertain overnight. One very good example is that of the invention of the laser which was an accidental discovery in the 1950s and not a lot of impact was felt or anticipated in the immediate future. However, the laser is a major success now and even nanomedicine research highly engages it.

Learning from past mistakes and listening to fellow researchers are imperative for scientific progress. The current trend of inclination toward only ‘positive data’ is harmful to many disciplines including nanomedicine because data are always positive as long as they add to the knowledge. Refusing publication/funding and ignoring the importance of experiments that did not deliver to the level of expectation are unhealthy practices. Science is never wrong and going back to basics (e.g., maintaining thorough lab notebooks and giving detailed honest methods while publishing) is the key to getting out of this crisis. It will only happen the day a humble generation of scientists like Frederick Sanger is back once more to mess about in the laboratory.

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