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No Nanobots in Vaccines — Just Lipids on the Loose:

Commentary on Lee and Broudy (2024), "Real-Time Self-Assembly of Stereomicroscopically Visible Artificial Constructs in Incubated Specimens of mRNA Products Mainly from Pfizer and Moderna: A Comprehensive Longitudinal Study"

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Summary

Lee and Broudy (2024) reported conspicuous microscopic objects in mRNA vaccines, which they interpreted as "*nano-robots*". This is a misconception, because the wide range of different shapes can be readily explained in terms of self-assembling lipids (including cholesterol), as are used for transfection. Lipid nanostructures and their rearrangements will be discussed.

Keywords: *cholesterol*, *Comirnaty, impurities in vaccines*, *lipid nanoparticles*, *mod mRNA transfection, Moderna*, *nanobots*, *nanorobotics*, *optical microscopy*, *Pfizer*, *self-assembling structures*, *transfection*

Background and Comments

Peculiar images of strangely shaped objects in mRNA vaccines are being reported not only in the social media, but now also in the scientific literature as in the recent *IJVTPR* publication by Lee and Broudy (2024) to which this comment is appended.

Several bewildering examples from their experimental analysis, using optical microscopy, are illustrated in Figure 1. The authors describe the observed objects as "*animated worm-like entities, disks, chains, spirals, tubes, right-angle structures containing other artificial entities within them*", and "*unique striated curled ribbons and various filaments, scaled like snakeskin, that seemed to contain hollow compartments as in bamboo*

shedding bubbles. These structures proliferated throughout the medium and were elongated, twisted, knotted, discolored, and varied in texture" (p. 1227).

As conveyed here and elsewhere, the angular platelets discovered in the COVID-19 injectables have a technical appearance that is reminiscent of microchips, the extended sheets and bubbles have been interpreted as graphene, and some of the soft ribbons and spirals look like parasites. Concerned readers and influencers are rightfully wondering whether these well documented phenomena are responsible for some of the injuries following vaccination? Or, as implied in said article, whether the manufacturer might even have covertly spiked the injections with "*nano-robots*" that can be "*programed*" to spring into action?

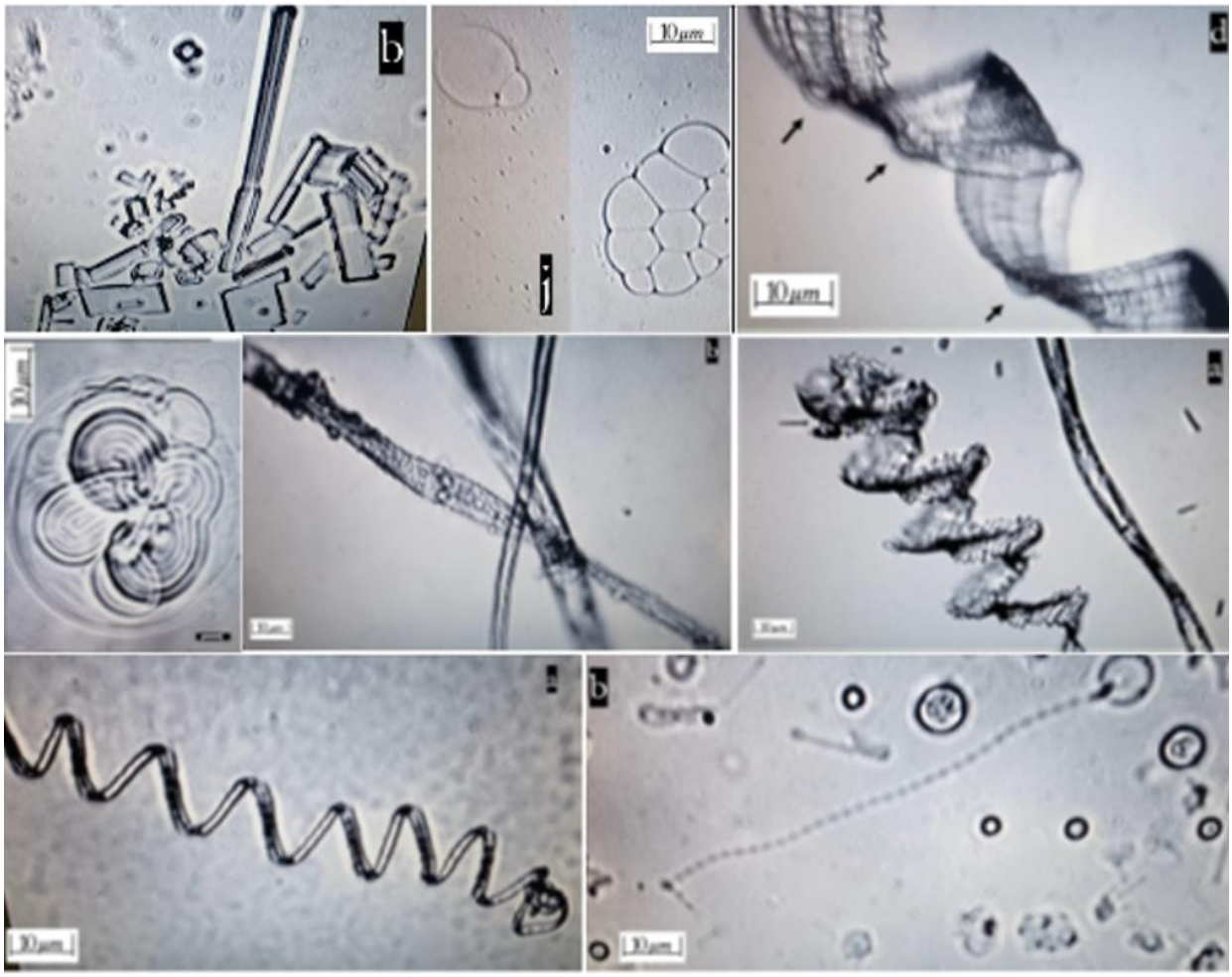

Figure 1. Overview of various representative microstructures observed in the COVID-19 vaccines from Pfizer/BioNTech and Moderna upon incubation. Copyright: Lee and Broudy (2024). Creative Commons License 4.0 NC ND.

Based on our scientific expertise and with full conviction, we would like to give reassurance that the abundant structures found in the mRNA vaccines are neither "*nanobots*" nor contaminants — but rather maturation and/or degradation products. This reasoning does not rule out the presence of minor impurities, as have been detected by highly sensitive analytical techniques, as also cited by Lee and Broudy. And it certainly does not give a thumbs-up for the new modRNA platform or a continuation of the vaccination campaign. However, it is important to differentiate between any

valid reasons for concern and other, falsely perceived dangers that arose from misinterpretation of imagery.

It has long been known in the field of biophysics that all of the intriguing geometries described here by Lee and Broudy (2024) can emerge naturally from lipid preparations, as are used in the modRNA vaccinations in the form of lipid nanoparticles. They may show up especially when samples are stored over extended periods — or under inappropriate conditions. The astonishing µm-sized architectures can be explained by a simple physical process called lipid self-assembly. Notably, lipids make up the bulk of the vaccine ingredients, in other words, 2.5 mg/ml (one quarter of which is cholesterol), compared to a mere 0.1 mg/ml modRNA, in the presence of about 100 mg/ml sucrose and salts (as declared for ready-to-use Comirnaty/BNT162b2). Therefore, instead of seeking an explanation for the strange objects in terms of exotic additives, it is more plausible to start looking at the materials that are already known to be abundantly present in the injectable formulations.

Here, we argue that the bizarre structures should not be considered alarming *per se*, as they are just made up of lipids. They clearly do not represent any allegedly "*toxic protein secretions*", "*long-lasting silica*", "*graphene-coated polymers*", "*conductors or semiconductors*" or any other "*undisclosed additional engineered components*" made up of "*not-natural/foreign material*". Nonetheless, it ought to be kept in mind that many of the lipid constituents used in the nanoparticles tend to induce inflammation (Chen & Blakney, 2024). They also have a high immunogenic and allergenic potential, which argues against an extensive application in humans. The intrinsic risks posed by the modRNA and its product, the spike protein, are yet another completely different matter that will not be addressed here. Instead, we shall look at the structure of lipid nanoparticles and explain their curious rearrangements into sheets, ribbons, filaments, spirals, tubes, chips, beads on a string, and much more.

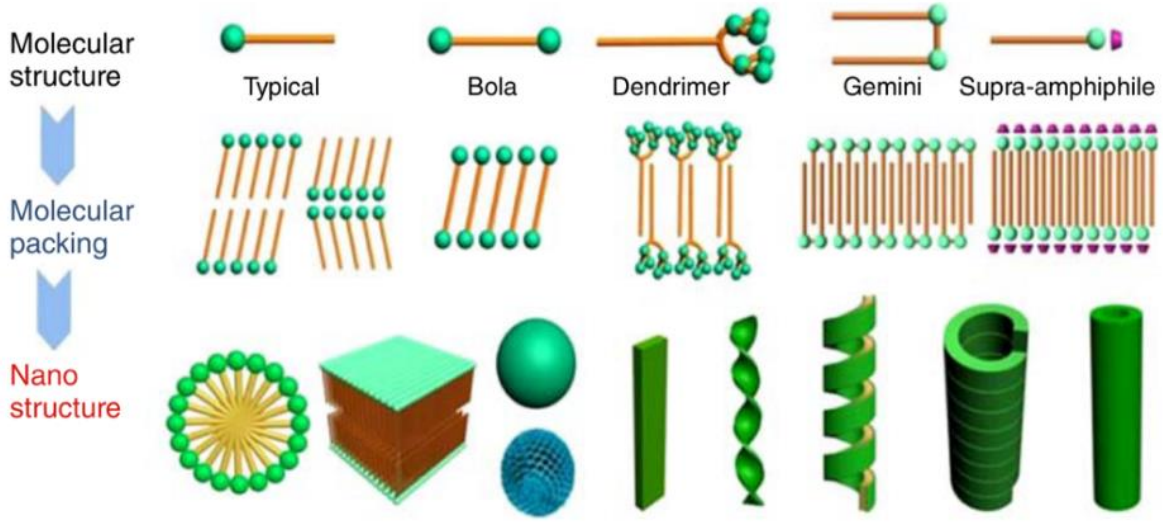

Figure 2. Self-assembly of typical lipid molecules (top row, left) into bilayers (middle row, left), which can produce a wide variety of different nanostructures (entire bottom row). Reprinted with permission from Jiang et al. (2018). Copyright 2024 by WILEY.

It is common knowledge that lipid mixtures are employed as delivery agents for the active ingredient, modRNA, in the injectables from Pfizer/BioNTech and Moderna. Amphiphilic lipid molecules are typically composed of a hydrophilic head and a hydrophobic tail, as illustrated at the top left of Figure 2, marked in green and yellow, respectively (from Jiang et al., 2018). In nature,

phospholipids and cholesterol (which is also a type of amphiphilic lipid) make up biomembranes, which envelope every cell. Lipid self-assembly happens spontaneously in water, as the amphiphiles nestle together side-by-side in a palisade, ending up tail-to-tail in a double layer (see "Molecular packing", Figure 2). The lipid membrane acts as a hydrophobic barrier between the aqueous interior of the cell and the outside world. Considering an isolated bilayer, any open hydrophobic edge would be unfavorable, which is why membranes tend to seal themselves into large hollow spheres, so-called vesicles. The bottom row of Figure 2 depicts the architectures of well-known nanostructures, from left to right: a tiny detergent micelle, a wafer-thin lipid bilayer, and a huge lipid vesicle built up from the latter (not to scale). Notably, as illustrated in the same row further towards the right, numerous other nanostructures are also possible, albeit not so common in biology — more on this below.

The new vaccine technology is based on solid lipid nanoparticles, which are not just hollow water-filled vesicles, but instead packed with modRNA and other special amphiphiles. The optimized nanoparticles contain on average one RNA molecule each, and their size of about 50 nm is well resolved by electron microscopy, as seen in Figure 3 (Unruh et al., 2024). Notably, each individual RNA chain carries over 4000 negative charges and has a nominal length of almost 3000 nm (using BNT162b2/Comirnaty from Pfizer/BioNTech as an example). In order to pack this long and sensitive RNA molecule snugly, it is bundled together with the help of cationic lipids. These designer-made synthetic amphiphiles (ALC-0315 from Pfizer/BioNTech, or SM-102 in mRNA-1273 from Moderna) take on a positive charge at low pH, and they bind well to anionic modRNA. Hence, they are located inside the nanoparticle, together with the modRNA and cholesterol. Each particle is enveloped by a monolayer of helper lipids, such as the natural membrane-forming phospholipid DSPC (distearoylphosphatidylcholine, similar to egg lecithin) and some cholesterol. As a final component, some PEGylated lipids with polyethylene-glycol chains are anchored to the surface (like ALC-0159 from Pfizer), to increase circulation time by acting as a protective water-soaked sponge.

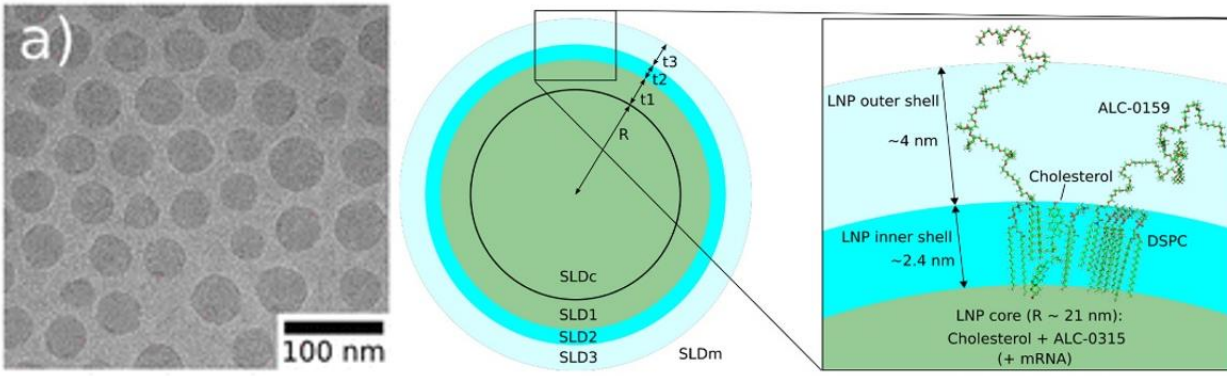

Figure 3. Injectable nanoparticles contain modRNA and cationic amphiphiles in the core, surrounded by a lipid monolayer and a PEGylated surface. Reprinted with permission from Unruh et al, (2024). Copyright 2024 American Chemical Society.

The task of the special lipid mixtures, also known as transfection agents, is to stabilize the sensitive RNA strands and to enable their delivery in the form of nanoparticles (Gote et al., 2023). Inside the body, the transfection agents serve as door openers when encountering any cell into which the modRNA is to be transported. Every cell membrane has special gates, which allow only certain substances to pass through, as are required by the cell or to be secreted. The uptake of mRNA is generally not intended, and free RNA gets rapidly degraded outside the cells. However, due to their amphiphilic nature, the lipid nanoparticles function like Trojan horses. By perturbing and fusing with

the cellular membrane (especially at low pH within an endosome), they can smuggle their cargo into the cell, where the modRNA will then be able to initiate the production of spike proteins. Voila!

Back to the current reports on the unusual microstructures and the alleged "*nanobots*" observed by Lee and Broudy (2024) in the vaccines. In fresh modRNA preparations or samples that have been stored properly, no objects should be visible under an optical microscope, because the original nanoparticles with a diameter of around 55 nm are well below the resolution limit of 200 nm (Unruh et al., 2024). According to the manufacturer, the unopened injectable suspensions are stable for up to 10 weeks in a refrigerator. However, if they are stored for longer times or at higher temperatures, or are subjected to freeze-thawing, dilution, agitation, different salt concentrations or pH values, they can break down progressively. The delicate RNA chains are generally sensitive to hydrolysis by acids, bases, bacteria, and enzymes (for example, trace amounts of ribonuclease on the human skin), as well as to oxidation by atmospheric oxygen. Lipids can also decompose chemically in similar ways. Recently, it has been demonstrated that the Pfizer/BioNtech and Moderna injectables are in fact quite stable towards physical stressors for up to 8 days. However, the cholesterol and RNA contained therein are photosensitive and tend to get oxidized under irradiation corresponding to bright sunlight (Fongaro et al., 2023). As a result of any such degradation process, the lipids will rearrange in new ways once they have lost their cohesion due to fragmentation of the RNA chains or due to any change in the molecular composition of the sample.

It is commonly accepted that the physical shapes and sizes of lipid nanoparticles (especially in combination with RNA or DNA) tend to be only meta-stable. This means that the particles are kinetically trapped and have not yet reached their lowest energy state thermodynamically. As a consequence, time-dependent changes in their morphological appearance would be expected simply upon letting them stand. It is important to note that Lee and Broudy (2024) not only incubated their material for extended periods of up to 12 months; but, in fact, they diluted some samples with distilled water or blood plasma. To other samples they added oxidants like H_2O_2 or ClO_2, or they mixed them with some electrolyte solutions, or various colloidal/mineral suspensions. Any changes in the aqueous environment, as well the addition of low molecular weight compounds, proteins or colloids, can dramatically affect the stability of nanoparticles. Under the diverse experimental conditions that were used, a regrouping of the lipids into other types of structures is not at all surprising.

A lipid mixture can often take on several different phase states and/or structural shapes, as is conveniently displayed in a phase-diagram, usually as a function of temperature and water content. But other parameters, too, have an influence of the phase behaviour of lipid systems, such as the component ratios, salt, pH, additives, pressure, etc., many of which will have been affected under the different experimental conditions. It is obvious that some molecular phase separation or de-mixing has taken place in some of the incubated vaccine samples, allowing the poorly water-soluble cholesterol to crystallize out. This interpretation can fully explain the appearance of the sharp-edged platelets and angular chips (top left corner of Figure 1), which have already been attributed to cholesterol crystals (or even simple salt) in earlier discussions cited by Lee and Broudy (2024).

The tendency of initially invisible but meta-stable nanostructures to transform into larger µm-sized agglomerates by fusion and/or aggregation has been well documented for many years. Such processes can therefore fully explain the images published by Lee and Broudy (2024). The structures in Figure 1 are typical of the morphological diversity of amphiphiles — phospholipids, cationic lipids, and cholesterol alike, as encountered here in the modRNA transfection mixtures. Figure 2 presents in the bottom row a veritable zoo of different self-assembled shapes, all of which have been recognized in biophysics for decades (Jiang et al., 2018). Some examples of interesting

geometries from the early days of lipid research are displayed in Figures 4 and 5, including various assemblies that are based on bilayers, as well as cholesterol crystals covered with a phospholipid monolayer. These images are highly reminiscent of the structures in Figure 1.

Based on the motif of a lipid bilayer and the need to minimize exposure of hydrophobic edges, conventional vesicles are most commonly formed, but hollow tubes and donuts have also been observed. Other remarkable descriptions include, for example, images of single vesicles that peal off from stacked multi-layers like blisters, groups of vesicles that assemble into foam-like bubbles, and spherical vesicles that have sprouted elongated tethers. Filaments, flat strips and ribbons can also emerge as transiet or long-lived structures, as long as their edges are covered by suitable amphiphiles. The underlying nano-sized structures can grow in length and width, get stacked, and continue to agglomerate. As soon as such assemblies reach a size of micrometers, they become visible under the optical microscope. Interestingly, it has been shown that flat ribbons have a tendency to twist into helices if they contain chiral (in other words, non-mirror symmetrical) molecular groups in their chemical framework (Singh et al., 1988). Such helices can curl up more and more tightly and eventually get sealed into tubes (see Figure 4, left). Tubes, in turn, have been seen to collapse into smaller partitions upon

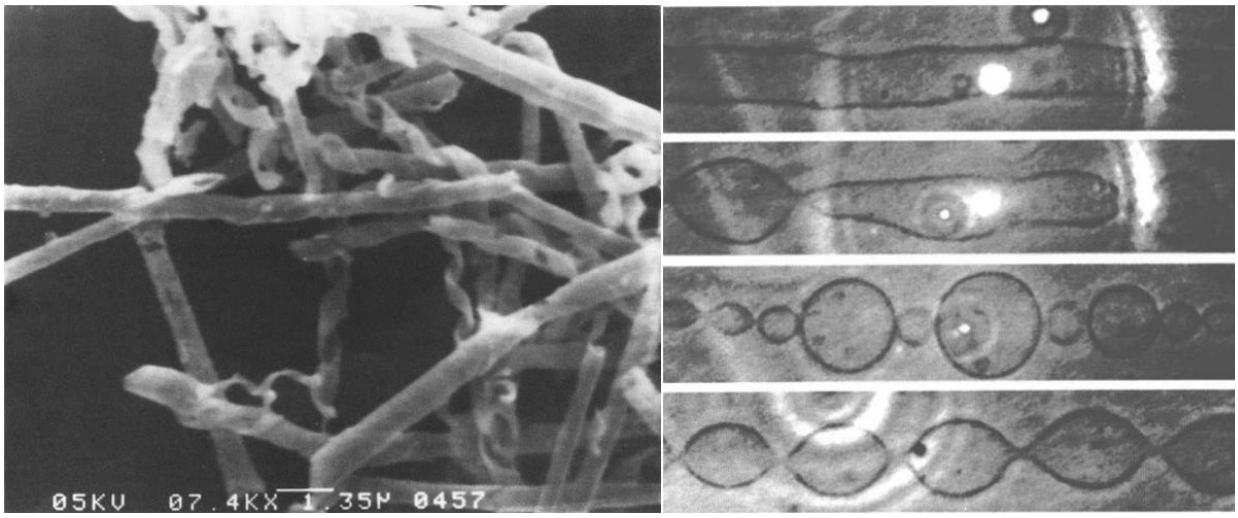

Figure 4. Early examples of unusual morphologies based on lipid bilayers. *Left*: Electron microscopy reveals helices and tubes that are assembled from a phospholipid (rac-$DC_{8,9}PC$ in isopropanol/water). Scale bar: 1.35 µm. Reprinted from Singh et al, 1988, Copyright 2024, with permission from Elsevier, conveyed by Copyright Clearance Center, Inc. *Right*: Time-lapse of a phospholipid tube (DMPC, with an original diameter of about 8 µm in the top panel) that is perturbed with optical tweezers. Reprinted figure with permission from Bar-Ziv and Moses (1994). Copyright 2024 by the American Physical Society.

external perturbation (for example, mechanical force, electromagnetic waves) and end up looking like beads on a string (see Figure 4, right). Interestingly, cholesterol crystals can also take on some of the aforementioned unusual shapes, when grown in the presence of phospholipids that can cover the hydrophobic crystal surfaces with a monolayer (Figure 5). Namely, in a model bile mixture, such enveloped crystals have been found to successively develop from filaments to springs, via helical ribbons and tubes, to eventually break up at the edges into angular fragments (Konikoff et al, 1992).

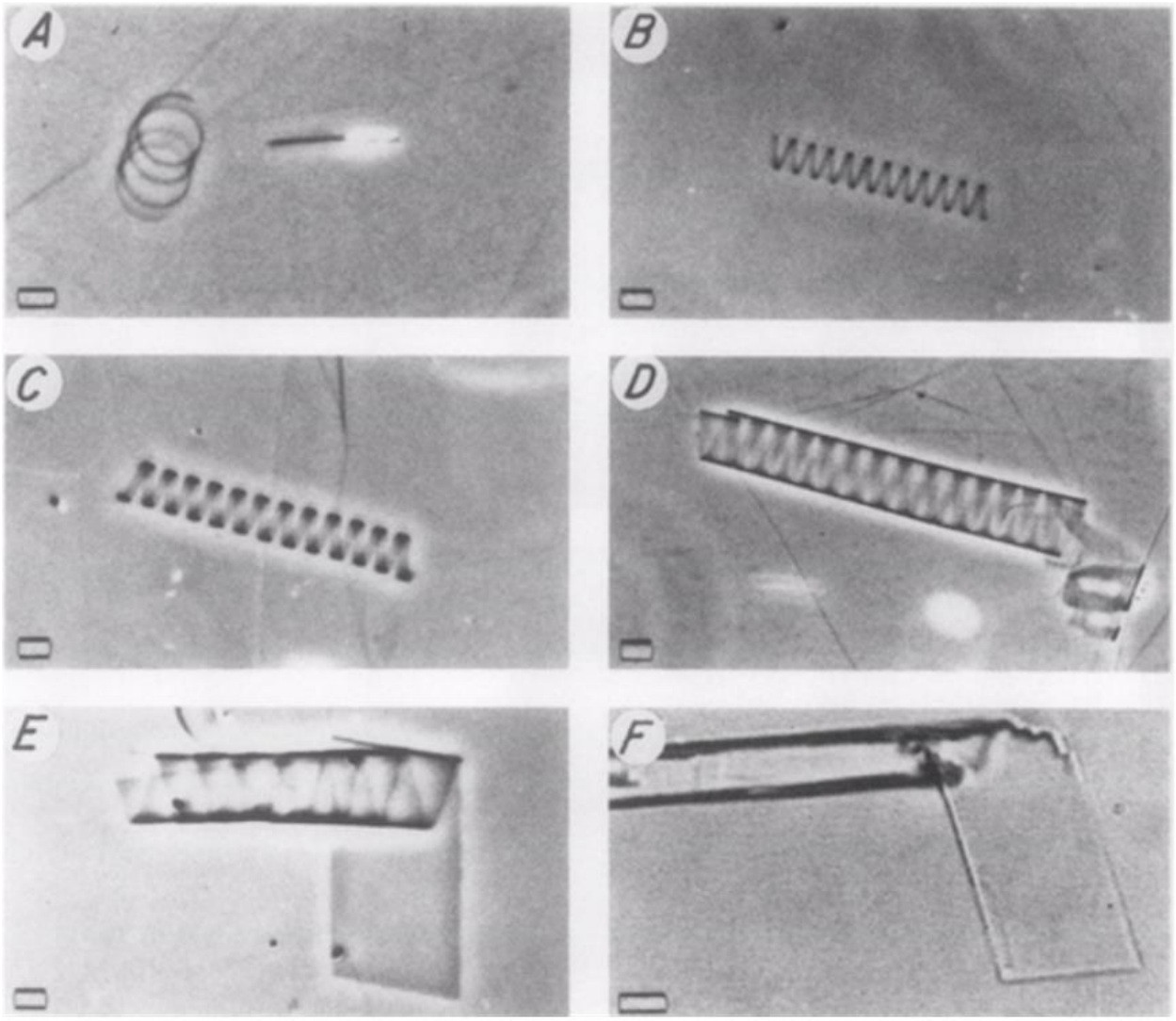

Figure 5. Helical cholesterol crystals, which are covered by a phospholipid monolayer, initially nucleate as filaments from a bile mixture (egg-lecithin:cholesterol 1:2 mol:mol, in excess taurocholate), then grow into tube-like structures, which eventually fracture at their ends to produce the characteristic angular cholesterol monohydrate platelets. Scale bars: 10 μm. Used with permission of the American Society for Clinical Investigation, from Konikoff et al, 1992. Copyright 2024, permission conveyed by Copyright Clearance Center, Inc.

All of the characteristic structures mentioned above are nicely illustrated and meticulously described in the current publication by Lee and Broudy (2024). Unfortunately, their lipidic origin has not been recognized, hence the images were profoundly misinterpreted. Their poignant description as "*synthetic hybrid organisms or possibly animated robotic structures*" that could be intentionally "*programed*" to respond to electromagnetic stimuli, is certainly not appropriate. The self-assembly of amphiphilic lipids (including cholesterol), which are contained at high concentrations in the modRNA injectables, is a purely passive and classical physico-chemical phenomenon.

Many aspects of the current publication suggest that the data and images are — in the light of our sobering interpretation — reliable and fully consistent, as the experiments were carried out diligently and the resulting images are well documented. For example, the angular plates were detected early on at the bottom of some culture dishes (Figure 1, top left corner), in full accordance with our interpretation as typical cholesterol crystals, whose density is greater than 1.0 g/ml. After about one

month, filaments, ribbons and coils were starting to emerge in the upper layers of the incubated vaccines samples, as well as beaded chains, near the surface of the solution (Figure 1, bottom right corner). These floating assemblies have a low density and should therefore be largely composed of phospholipids and cationic amphiphiles. Most likely, the conspicuous helices in Figure 1 are composed of both cholesterol and lipids (phospholipids and/or cationic amphiphiles).

The observation that virtually no bacteria appear to grow in the mRNA vaccines even after long incubation periods (a small amount of protein was nonetheless found) confirms the notion that cationic amphiphiles often have antimicrobial effects. That is because positively charged amphiphiles or lipids (note that cationic lipids do not usually occur in nature) bind to and destroy the anionic lipid membranes of bacteria. Human cells carry significantly fewer negative charges on their surface. Therefore, they are not as readily damaged by cationic amphiphiles, although some toxicity is to be expected at high concentrations. Fortunately, the new generation of ionizable (in other words: pH-dependent) cationic lipids used by Pfizer/BioNTech and Moderna tend to be less cytotoxic than traditional transfection agents consisting of permanently charged cationic amphiphiles. A perturbation of human cells, however, was nonetheless demonstrated in the present study by Lee and Broudy, when examining red and white blood cells, platelets, and sperm. When, for example, the vaccines were added directly to blood, the erythrocytes became deformed or stacked together like rolls of coins, called rouleaux.

It should be borne in mind that an injection of the highly concentrated vaccines into the deltoid muscle would initially cause only a local disruption of cells and inflammation, but some nanoparticles can be taken up by the lymph or blood and become distributed throughout the body. Due to the resulting dilution, the primary cytotoxicity issues should be less of a concern (though the zeta-potential in the affected bodily fluids may be reduced). On the other hand, transfection and expression of spike protein in the blood vessels or other sensitive tissues can have disastrous immunological consequences. After all, a fresh dose of vaccine contains around 10^{13} modRNA molecules, which corresponds to 10 trillion nanoparticles administered. Just for comparison, this order of magnitude matches roughly the number of cells in a human body, so the organism is literally flooded with nanoparticles.

In summary, there is no reason to be afraid of "*nanobots*" in vaccines. It should nevertheless be clear that the new generation of modRNA products entails considerable risks, not so much due to the toxicity of the lipids, but rather due to the genetically active components they deliver. Even in the light of potentially harmful lipids, the amounts administered are essentially under control, and their cytotoxic activity can be assessed, as is commonplace with other traditional pharmaceuticals. Expression of the spike protein, on the other hand, is fundamentally beyond control with regard to the distribution of the lipid nanoparticles, the amount of modRNA that is taken up by any cell and reaches the cytoplasm, the tissue type that gets transfected, the persistence of protein expression, the lifetime of active modRNA, its potential incorporation into DNA, and any long-term effects on the immune system. These risks will be exacerbated with the next generation of self-amplifying or self-replicating RNA vectors for vaccines. In our opinion, further research and public discussion should focus on these critical aspects, rather than stirring up excessive fear of futuristic, transhumanist manipulation through nanotechnology. It appears that there occurred a naïve and premature misinterpretation of the data here, which should hopefully be resolved soon within the scientific community in accordance with good publication practice. Yet, the present example shows

once again how the anxious reception of an unexpected image can shift the bias towards a worst-case scenario — be it a lipid structure under the microscope or a convoy in Bergamo.[1]

Conflict of Interest Statement

I declare that I have no conflict of interest with regard to any of the content of the manuscript. I am employed solely by the Karlsruhe Institute of Technology (KIT), as a full professor of biochemistry. The contributed manuscript is not of an experimental nature, but purely an intellectual excerpt of my working experience with membrane biophysics over the last 35 years, so no particular external funding has been acquired to derive these results.

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[1] **Editor's Note:** For future reference, this last phrase, "a convoy in Bergamo", refers to an event captured first in a single cell-phone photo of a late night scene in the city by that name in Northern Italy on March 18, 2021. The Associated Press at the hyperlink here described it as ". . . one of the most haunting moments of the pandemic: when Bergamo's death toll reached such heights that an army convoy had to transport coffins out because its cemeteries and crematoriums were [allegedly] full". The legend born that night is described by Julie Metzdorf here. From "di Terlizzi's cell phone [the] photo from Bergamo", she says, "was what we like to call 'authentic' today: not staged, it depicts the nighttime scene as it was . . . . A military convoy driving through a residential area at night: . . . In truth, the military was not deployed because there was no other way to deal with mountains of corpses [of people who supposedly fell prey to SARS-CoV-2]. . . . [In actuality, Metzdorf says] the number of deceased at that time was no higher than during some flu waves in Italy (as of April 2020). It was the fear of the pathogen known as the 'killer virus'. . . . [that led to the perceived urgency to] immediately cremate those who died of COVID. Normally, however, only half of all deceased people are cremated in Italy. Therefore, the capacity of the crematorium in Bergamo was not sufficient and the bodies had to be transported to surrounding towns." Even though the "mountains of corpses" were only imagined, the fear created throughout Europe was real.

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