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mRNA Purifications Process: Affinity Chromatography, Magnetic Beads and Graphene Coated Large Scale Production and Toxicological Aspects

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Abstract

Aim of this work is to verify the state of the art related m RNA purification process, the technology used as well as the materials employed. New methods vs classic methods: the use of affinity chromatography or and high-gradient magnetic separation (H.G.M.S.). In particular the use of magnetic beads since introduction of the graphene coated ones. Various producers provide much kind of magnetic beads with various grade of efficiency in separation. Because today there is a great debate around graphene derivate presence (or not) in vials of m RNA vaccine it is relevant to better clarify the production process as well and the materials used. This is of toxicological interest.

Keywords: m RNA vaccine • Production • Purification • Affinity chromatography • Magnetic beads • Magnetic beads graphene coated • Biopharmaceutical • Large scale

Introduction

"Down-stream processing needs more innovative ideas to advance and overcome current bio-processing challenges. Chromatography is by far the most prevalent technique used by a conservative industrial sector. Chromatography has many advantages but also often represents the most expensive step in a pharmaceutical production process. So, alternative methods as well as further processing strategies are urgently needed. One promising candidate for new developments on a large scale is the magnetic separation, which enables the fast and direct capture of target molecules in fermentation broths. There has been a small revolution in this area in the last 10–20 years and a few papers dealing with the use of magnetic separation in bio-processing examples beyond the analytical scale have been published. Since each target material is purified with a different magnetic separation approach, the comparison of processes is not trivial but would help to understand and improve the magnetic separation and thus making it attractive for the technical scale. We report on the latest achievements in magnetic separation technology and offer an overview of the progress of the capture and separation of bio molecules derived from biotechnology and food technology. The Magnetic separation has great potential for high-throughput down-stream processing in applied life sciences. At the same time, 2 major challenges need to be overcome: (1) the development of a platform for suitable and flexible separation devices and (2) additional investigations of advantageous processing conditions, especially during recovery. Concentration and purification factors need to be improved to pave the way for the broader use of magnetic applications. The innovative combination of magnetic gradients and multipurpose separations will set new magnetic-based trends for large scale downstream processing. Magnetic-separation is an interesting candidate for future down-stream applications due to some important advantageous features:

1. Integrated 1 step capture and purification of target (high affinity and selectivity)
2. High throughput
3. Semi-continuous processing with low energy consumption.
4. Thus, magnetic separation can help reduce costs and increase yields and productivity compared to traditional processes."

"The webinar will review the basic concepts of magnetic -separation and help the attendees understand how advanced systems may enlight key aspects of the process. These concepts will be applied to parameterize, monitor and validate the magnetic beads behavior in controlled conditions. Afterwards, the discussion will focus on how to transfer the correctly characterized bio-magnetic separation process from laboratory to production scale." (Figure 1)

"Large Scale DNA and RNA Oligonucleotide Production Services

Synthesis: Solid-phase synthesis has been employed for the manufacture of oligonucleotides from micrograms for research use. During the solid-phase synthesis, phosphoramidite monomers are added sequentially onto a solid support to generate the desired full-length oligonucleotide. Each cycle of base addition consists of 4 chemical reactions, detritylation, coupling, oxidation/thiolation, and capping.

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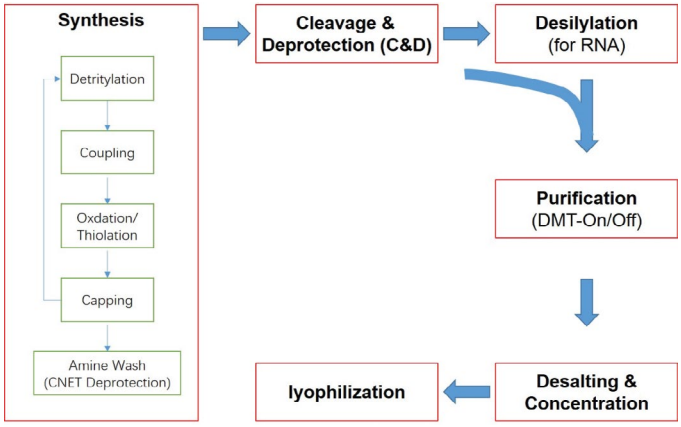

Figure 1. Oligonucleotide production process steps.

Cleavage and Deprotection (C&D): Oligo-nucleotide de-protection involves 3 steps: removal of a cyanoethyl protecting group from the phosphate backbone, cleavage of the oligonucleotide chain from the support, and base deprotection. This process is generally carried out in batch mode where the entirety of the solid support is incubated with C&D reagents.

Purification: The purification of oligonucleotide crude solutions is generally achieved by chromatographic methods. Taking advantage of the inter nucleotide phosphodiester and phosphorothioate linkage, anion exchanged and ion-paired reversed-phase (IP-RP) chromatographic purifications on HPLC equipment are widely used for the purification of the therapeutic oligonucleotide.

Desalting and Concentration: The most widely accepted choice for therapeutic oligonucleotide isolation at manufacturing scales is the use of tangential flow filtration (TFF). This approach employs an appropriately sized filter membrane and uses a pump to circulate the sample through the TFF set-up making the process more efficient.

Lyophilization: This step is performed using a freezer dryer, which is a machine consisting of a sample chamber, a condenser and a vacuum pump. The oligonucleotide is frozen and lyophilized to obtain the final product in powder form via sublimation." (Figure 2-4)

Pre activated Magnetic Beads for Affinity Chromatography

NHS Mag Sepharose, Sera-Mag Carboxylate-Modified, Sera-Mag Speed beads Carboxylate-Modify. "Pre activated magnetic beads are designed for covalent coupling of antibodies, aptamers, and proteins. After coupling is performed, proteins of interest can be affinity captured and enriched using immuno precipitation. The pre activated magnetic beads include NHS Mag Sepharose, Sera-Mag Carboxylate-Modified, and Sera-Mag Speed beads Carboxylate-Modified Magnetic Particles".

"Magnetic beads are used to purify single proteins, large protein complexes, antibodies and for high-throughput purifications. Magnetic beads based on chromatography resins provide functions of the resin with the convenience and ease-of-use of magnetic beads".

"Ribonucleic acid purification disclosed herein is methods for purifying RNA comprising poly A. Also disclosed herein do compositions such as surfaces and oligonucleotides for purifying RNA comprise poly A. Other embodiments are also disclosed. Commercially-available resins having polythymidine oligonucleotide ligands typically contain less than 30 thymidine (2'deoxy) residues and some commercial resin suppliers utilize a distribution of dT chain lengths, not of a discreet length. Commercially-available matrices typically consist of cellulose, latex particles, and magnetic beads containing dT ligands".

"Purification of m R.N.A. with oligo (dT)-functionalized magnetic particles involves a series of magnetic separations for buffer exchange and washing. Magnetic particles interact and agglomerate with each other when a magnetic field is applied, which can result in a decreased total surface area and thus a decreased yield of mR.N.A.. In addition, agglomeration may also be caused by mR.N.A. loading on the magnetic particles. High-gradient magnetic separation (H.G.M.S.) can be used to selectively separate magnetizable components from suspensions. This technique has already been applied by various working groups in the field of biotechnology as well. It was utilized for the separation of immobilized enzymes and used for the isolation of target molecules. In this process, the target molecule is specifically adsorbed on the functionalized particle surface in a reaction mixture and desorbed again from the magnetic particles (named magnetic beads) after magnetic separation has taken place. An overview of widespread magnetic separators and magnetic particle systems is given in. One widely used micro particle system in the bio separation of proteins, m R.N.A., and viruses is Dyna beads pharmacy is the production of mR.N.A. for vaccine manufacturing. The synthesis of an mR.N.A. vaccine is described in the literature. Within the production of a vaccine, separation and purification of m R.N.A. are necessary in the process."

Practical Application

Magnetic micro particles have gained importance in the purification of mR.N.A.-based vaccines. They serve as adsorbents for the m R.N.A. Through several steps of magnetic separation followed by redispersion of the magnetic beads for washing and elution, the mR.N.A. can be isolated. This is usually done on an mL scale. To obtain larger amounts of m R.N.A., flow-through magnetic separation using high-gradient magnetic separation (H.G.M.S.) can be advantageous. Here, the suspension to be processed is then usually in a stirred feed tank. Due to particle-particle interactions, a not inconsiderable agglomeration may occur, especially due to the attached mR.N.A.. This can reduce the mR.N.A. yield. For economic reasons it is necessary to perform magnetic particle recycling, for which there are various process alternatives. With regard to a possible use of H.G.M.S. in an m R.N.A. production for vaccines, particle size distributions were determined to investigate the agglomerating or deagglomerating effect of different process steps."

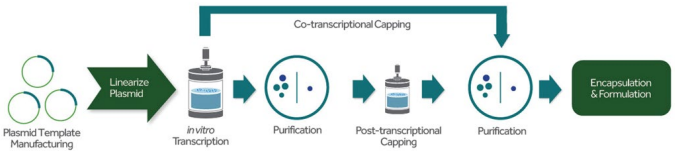

Figure 2. Purification process.

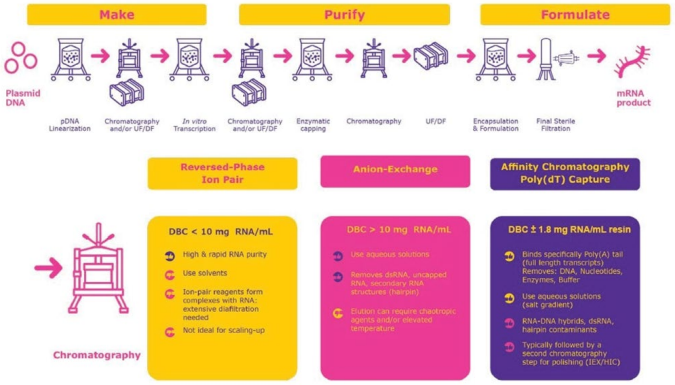

Figure 3. Types of chromatography.

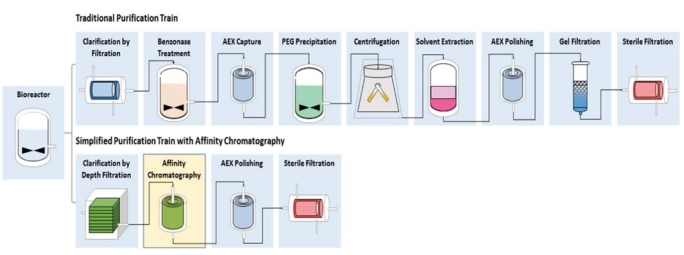

Figure 4. Traditional purification train.

"A specialized graphene oxide G.O hydro-gel can help stabilize therapeutic mR.N.A. cancer vaccines and release them slowly into the target tissue, show early results from the National Center for Nano-science and Technology in Beijing."

Materials and Methods

Related the topics of this work various relevant reference are reported, all article comes from PUBMED or other scientific database. After this review part and experimental project hypothesis is submitted to the researcher in order to provide a global conclusion related to the aim of the work.

Results

From literature

"Therapeutic cancer vaccines require a -robust cellular immunity for the efficient killing of tumor cells, and recent advances in neo-antigen discovery may provide safe and promising targets for cancer vaccines. Elicitation of T cells with strong antitumor efficacy requires intricate multistep processes that have been difficult to attain with traditional vaccination approaches. A multifunctional nano vaccine platform has been developed for direct delivery of neo-antigens and adjuvants to lymph nodes (LNs) and highly efficient induction of neo antigen-specific T cell responses. A PEGylated reduced graphene oxide nano sheet (R.G.O.-PEG, 20-30 nm in diameter) is a highly modular and biodegradable platform for facile preparation of neoantigen vaccines within 2 h. R.G.O.-PEG exhibits rapid, efficient (15-20% ID/g), and sustained (up to 72 h) accumulation in LNs, achieving >100-fold improvement in LN-targeted delivery, compared with soluble vaccines. R.G.O.-PEG induces intracellular reactive-oxygen species in dendritic cells, guiding antigen processing and presentation to T cells. Importantly, a single injection of R.G.O.-PEG vaccine elicits potent neoantigen-specific T cell responses lasting up to 30 days and eradicates established MC-38 colon carcinoma. Further combination with anti-PD-1 therapy achieved great therapeutic improvements against B16F10 melanoma. R.G.O.-PEG may serve a powerful delivery platform for personalized cancer vaccination." [1]

"Messenger RNA (m R.N.A.) vaccine is a promising candidate in cancer immunotherapy as it can encode tumor-associated antigens with an excellent safety profile. Unfortunately, the inherent instability of RNA and translational efficiency are major limitations of RNA vaccine. We report an injectable hydro-gel formed with graphene oxide (GO) and polyethylenimine (PEI), which can generate m R.N.A. (ovalbumin, a model antigen) and adjuvants (R848)-laden nano vaccines for at least 30 days after subcutaneous injection. The released nano vaccines can protect the mR.N.A. from degradation and confer targeted delivering capacity to lymph nodes. The data show that this transformable hydro-gel can significantly increase the number of antigen-specific CD8+ T cells and subsequently inhibit the tumor growth with only 1 treatment. This hydro-gel can generate an antigen specific antibody in the serum which in turn prevents the occurrence of metastasis. Collectively, these results demonstrate the potential of the PEI-functionalized GO transformable hydro-gel for effective cancer immuno-therapy." [2]

"Gene therapy is emerging as a valid method for the treatment of ovarian cancer, including small interfering RNA (siRNA). Although it is so powerful, few targeting efficient gene delivery systems seriously hindered the development of gene therapy. In this work study, we synthesized a novel gene vector PEG-GO-PEI-FA by functionalized graphene oxide (G.O), in which folic acid can specifically bind to the foliate receptor (FR), which is overexpressed in ovarian cancer. Characterizations of the nano complexes were evaluated by dynamic light scattering (DLS), atomic force microscopy, and Fourier transform infrared spectroscopy (FTIR). The siRNA condensation ability and stability were assessed by agarose gel electrophoresis. Cellular uptake efficiency and lysosomal escape ability in ovarian cancer cells were investigated by confocal laser scanning microscopy. Cellular bio safety of the system and inhibitory of the siRNA tolerability were evaluated by CCK-8 assay. The size of the PEG-GO-PEI-FA nano complexes was 216.1 ± 2.457 nm, exhibiting mild cytotoxicity in ovarian cancer cells. With high uptake efficiency, PEG-GO-PEI-FA can escape from the lysosome rapidly and release the gene. PEG-GO-PEI-FA/siRNA can effectively inhibit the growth of ovarian cancer cells. By and large, the PEG-GO-PEI-FA/siRNA may offer a promising strategy for siRNA delivery in the treatment of FR-positive ovarian carcinoma or similar tumors." [3]

"Isolated, atomically thin conducting membranes of graphite, called graphene, have recently been the subject of intense research with the hope that practical applications in fields ranging from electronics to energy science will emerge. The atomic thinness, stability and electrical sensitivity of graphene motivated us to investigate the potential use of graphene membranes and graphene nano pores to characterize single molecules of D.N.A. in ionic solution" Electrical measurements on graphene membranes in which a single nano pore has been drilled show that the membrane's effective insulating thickness is less than 1 nano-meter. This small effective thickness makes graphene an ideal substrate for very high-resolution, high throughput nano pore-based single molecule detectors. [4]

Metrics: "Graphene Substrates for Drug Delivery GO with its oxygen-containing functional groups (COOH and OH) has been reported as an effective carrier for drug or gene delivery." [5]

"Clinical applications of induced pluripotent stem cells require development of technologies for the production of "footprint-free" (gene integration-free) iPSCs, which avoid the potential risk of insertion mutagenesis in humans. Previously, several studies have shown that mR.N.A. transfer can generate "footprint-free" iPSCs, but these studies did not use a delivery vehicle and thus repetitive daily transfection was required because of mR.N.A. degradation. We report an mR.N.A. delivery system employing graphene oxide (GO)-polyethylenimine complexes for the efficient generation of "footprint-free" iPSCs. GO-PEI complexes were found to be very effective for loading mR.N.A. of reprogramming transcription factors and protection from mR.N.A. degradation by RNAse. Dynamic suspension cultures of G.O-PEI/RNA complexes-treated cells dramatically increased the reprogramming efficiency and successfully generated rat and human iPSCs from adult adipose tissue-derived fibroblasts without repetitive daily transfection. The iPSCs showed all the hallmarks of pluripotent stem cells including expression of pluripotency genes, epigenetic reprogramming, and differentiation into the 3 germ layers. These results demonstrate that mR.N.A. delivery using GO-PEI-RNA complexes can efficiently generate "footprint-free" iPSCs, which may advance the translation of iPSC technology into the clinical settings." [6]

"The observations under a phase contrast, Dark-Field, Bright Field microscopy, Transmission and Scanning Electron microscopy of the vaccine product by Pfizer, including vaccine products of Modern a, Astrazeneca and Janssen revealed some entities that can be graphene strips are shown. Optical phase contrast microscopy, 600X. The Muestra RD1, La Quinta Columna Report, June 28, 2021; Graphene Oxide Detection in Aqueous Suspension; Delgado Martin, Campra Madrid confirmed our findings]. For a definitive identification of graphene by TEM, it is necessary to complement the observation with the structural characterization by obtaining a characteristic electron diffraction standard sample. The standard sample corresponding to graphite or graphene has a hexagonal symmetry, and generally has several concentric hexagons. Using Transmission Electron Microscopy (TEM) we observed an intricate matrix or mesh of folded translucent flexible R.G.O. sheets with a mixture of darker multilayer agglomerations and lighter colored of unfolded monolayers as seen. This shows the liposome Capsid containing R.G.O. that Pfizer uses for its product to reticulate the graphene oxide by attaching the Liposome capsid to specific mR.N.A. molecules for driving the Liposome contents of fGO to specific organs, glands and tissues, namely the ovaries and testes, bone marrow, heart and brain. The image was obtained by a SEM-Cryo preparation.] Using (TEM) revealed an intricate matrix or mesh of folded translucent flexible R.G.O. sheets with a mixture of darker multilayer agglomerations and lighter colored of unfolded monolayers as seen. This shows a cluster of graphene nano-particles in a Pfizer vaccine. They appear to be aggregated. The darker linear areas in (Figure 5) reported appear to be local overlap of sheets and local arrangement of individual sheets in parallel to the electron beam. After the mesh, a high density of unidentified rounded and elliptical clear shapes appears, possibly corresponding to holes generated by

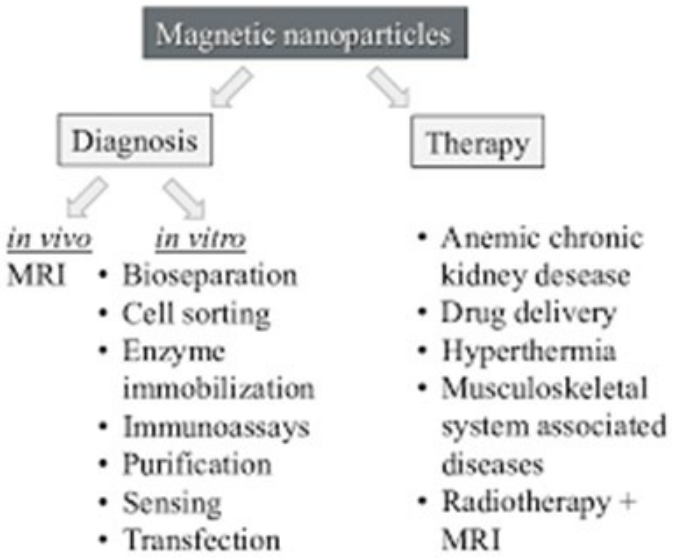

Figure 5. Magnetic nanoparticles.

mechanical forcing of the R.G.O. mesh during treatment as seen. The X-ray diffractometer reveals their nature of crystalline Carbon-based nano-particles of R.G.O. Energy-Dispersive X-ray Spectroscopy Reveals R.G.O. in Pfizer Vaccine. The Pfizer vaccine liquid fraction was then analyzed for chemical and elemental content using Energy-dispersive X-ray spectroscopy (E.D.S.) as seen. The E.D.S. spectrum showed the presence of Carbon, Oxygen verifying the R.G.O. elements and Sodium and Chloride since the sample shown reported was diluted in a saline solution. The quantification of m R.N.A. in the Pfizer Vaccine The quantification of RNA in the Pfizer sample was carried out with conventional protocols (Fisher). According to NanoDropTM 2000 spectro photometer calibration check specific software (Thermo fisher), the UV absorption spectrum of total aqueous fraction was correlated to 747 ng/ul of unknown absorbing substances. After RNA extraction with commercial kit (Thermofisher), quantification with RNA specific Quit fluorescence probe (Thermofisher) showed that only 6t ug/ul could be related to the presence of RNA." [7]

"The invention belongs to the field of nano materials and biological medicines, and relates to a vaccine, in particular to development of a 2019-nCoV coronavirus nuclear recombinant nano vaccine. The invention also comprises a preparation method of the vaccine and application of the vaccine in animal experiments. The novel coronavirus vaccine contains graphene oxide, carnosine, CpG and novel coronavirus RBD; The carnosine, the CpG and novel coronavirus RBD are combined on a framework of the graphene oxide; the coding sequence of the CpG is as shown in SEQ ID NO 1; and the novel coronavirus RBD refers to that a novel coronavirus protein receptor binding region can generate a high-titer specific antibody aiming at the RBD in a mouse body, and strong support is provided for prevention and treatment of the novel coronavirus." [8]

"A magnetic material that consists of silica-coated magnetic beads conjugated with graphene oxide (GO) was successfully prepared for facile ribonucleic acid (RNA) extraction. When the G.O-modified magnetic beads were applied to separate the RNA from the lysed cell, the cellular RNAs were readily adsorbed to and readily desorbed from the surface of the G-O-modified magnetic beads by urea. The amount of RNA extracted by the GO-modified magnetic beads was about 170% as much as those of the control extracted by a conventional phenol-based chemotropic solution. These results demonstrate that the facile method of RNA separation by using G.O-modified magnetic beads as an adsorbent is an efficient and simple way to purify intact cellular RNAs and/or microRNA from cell lysates. Â© 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim". [9]

Google Patent: "The invention relates to a process for separating a dispersed phase from a continuous phase comprising the steps of i) contacting said phases with an effective amount of nano-particles; ii) applying a magnetic field gradient to the obtained system; iii) separating the obtained phases wherein said nano-particles are of the core shell type, said core consists of a metal or alloy having soft magnetic properties and said shell contains a graphene layers which are optionally functionalized; to new nano-particles and method of manufacturing such nano-particles. In yet another application e.g, for food additive production or in biotechnical production of proteins, viruses, vaccines and antibodies the magnetic functionalization of the cells may be used to keep them afloat in a desired volume of liquid allowing the separation of the liquid, containing a desired product from their site of production (cells, microorganisms). This application may also be applied in waste-treatment plants."

"A new variant of vaccine production is becoming increasingly important with regard to viral infectious diseases. This involves the use of mR.N.A. produced by cell culture methods. This m R.N.A. is protected by special formulations and introduced into human cells, where it induces the expression of proteins and thus, triggers the immune response. In the search for new vaccines, lab. Protocols that use functionalized magnetic beads for the purification of mR.N.A. out of cell lysates are often used. For this purpose, magnetic particles with deoxythymidine functionalization (oligo (dT)), with a sequence of 14–25 thymine bases are utilized. Hybridization of the m R.N.A. to the oligo (dT) magnetic particles takes place specifically, due to the base adenine complementary to thymine. Only m R.N.A. molecules have an adenine chain with 40–250 units at the 3′ end. This does not exist on RNA or D.N.A. molecules, so selective sorption occur by hybridization of mR.N.A. on the oligo (dT) magnetic particles. After separation of the particles loaded with m R.N.A. in the magnetic field and removal of impurities with various washing steps, elution of the mR.N.A. can be initiated by increasing the temperature. Here, successive multiple magnetic separations and suspensions are involved, for which suitable milliliter scale lab. Protocols have been developed, some of which are automated. With the developed SU-H.G.M.S. separation chamber, mR.N.A. manufacture can be realized at such a production scale, which should be sufficient for clinical trials. The standard operating procedures can be transferred directly from the corresponding lab. Protocols using magnetic particles. Increasing the amount of magnetic particles to be processed can be easily achieved by parallelization (up-numbering). High-gradient magnetic separation. If it was possible to use oligo (dT) magnetic particle-based purification of mR.N.A. for clinical trials, there would be no need for a technology change in scale-up from lab scale to process scale. With high-gradient magnetic separation (H.G.M.S.), the required process technology is available. It has already been used by various working groups in the field of bio technology. The magnetic separators used at H.G.M.S., which operate in the flow-through mode ("magnetic filters"), were and are developed in particular by Franzreb and in own work. The advantage of this technique in the production of m R.N.A. arises from the fact that the laboratory protocols used by several users in research can be transferred 1:1 to production." [10]

"Several novel magnetic beads are also currently in development, such as Quick Gel™ beads from Quad Technologies (US), new "big beads" from Cell Cap Technologies Ltd (UK), and metallic beads from Turbo Beads LIC (Switzerland). Many of the innovative efforts attempt to challenge the current paradigm of iron oxide beads (50 nm – 10 μm in diameter) that remain attached to the cell surface. Quick Gel™ beads are synthesized from a patented hydro-gel technology that allows for facile release of the beads from the cell surface. Cell Cap beads possess diameters in excess of 50 μm thus their operation relies on gravitational forces combined with magnetic forces to separate labeled cells from suspension. Turbo Beads® possess a magnetic metal core and a graphene shell of monolayer thickness; they thus present a high moment and stable labeling potential. Overall, magnetic cell separation is a growing industry and shows much promise for continued future innovations." [11]

"Although AC has rarely been incorporated in industrial vaccine purification, ion exchange and hydrophobic interaction chromatography (HIC) columns have been widely used in manufacturing-scale processes in combination with other separation techniques such as filtration, precipitation and ultracentrifugation. Affinity chromatography is among the most powerful separation techniques, achieving the finest separation with high yields even in the most challenging feed streams. Incorporating affinity chromatography

in vaccine purification has long been attempted by researchers to improve unit yield and purity with the secondary goal of reducing the number of downstream process operations. Despite the success in laboratory-scale proof of concept, implementation of this technique in pilot or cGMP manufacturing has rarely been realized due to technical and economic challenges in design and manufacturing of ideal ligands as well as availability of high-productivity chromatography media. This work paper reviews evolving technologies in engineered ligands and chromatography media that are encouraging companies to re-visit the possible use of affinity chromatography in larger scale vaccine purification. It is postulated that commercial-scale implementation of high throughput single-use affinity chromatography can significantly simplify process architecture, improve productivity and flexibility, and reduce cost of goods. Triple-helix, peptide, and amino acid-D.N.A. affinity chromatography have been studied for p D.N.A. purification. RNA and D.N.A. aptamers have been reported to recognize vaccine virus, HIV, HCV, and influenza virus." [12] (Figure 5)

"The invention is directed to compositions and methods for rapidly and efficiently extracting nucleic acids and/or targeted nucleic acids sequences from biological samples. The methods of the invention comprise combining the sample with a buffer and magnetic silicon beads and concentrating the beads with a magnet or other electrical field. Liquid may be removed, or not, and an alkaline buffer is added followed by magnetic carboxy beads in a binding buffer so that nucleic acids transfer to the carboxy beads, which can be easily and quickly isolated once again with a magnet. Total nucleic acid extraction is greatly enhanced. Extracted nucleic acids can be analyzed, for example, by PCR wherein the nucleic acids can be identified and characterized. Carboxy beads may also contain a ligand so as to target specific nucleic acid sequences. The invention is also directed to kits comprising the tools and compositions for performing the methods of the invention.

Exemplary nucleic acid capture matrix (NACM) materials include, preferably, agarose, glass, cellulose, polyacrylamide, Sepharose, Sephadex, silica, or another matrix media. Preferably, the NACM material is coated with a nucleic acid binding substance (NABS), such as, for example, nucleic acid (NA) binding proteins, antibodies and chemicals with an affinity for NAs including single-stranded nucleic acid sequences. NACM include materials coupled to specific antibodies or antibody fragments or other nucleic acids or ligands that facilitate extract and/or isolation of the diagnostic molecule of interest. Affinity beads are preferably magnetic beads such as, for example, beads commercially available from Dyna beads®, Life Technologies; Turbo Beads, Turbo Beads Inc. or Pure Proteome™, and Millipore."

"Nowadays, a huge variety of magnetic beads featuring a large diversity for different applications is commercially available. Just to name a few: Dyna beads® Magnetic beads provided by Invitrogen, Estapor® Super Paramagnetic Microspheres and Pure Proteome™ Magnetic Beads by Merck Millipore, Bc Mag™ by Bioclone Inc., Pro Mag™ and Bio Mag® from Bangslabs, Supra Mag™ by Poly microspheres Inc., Turbo Beads® by Turbo beads Llc., and SPHERO™ Polystyrene Magnetic Particles by Spherotech. Other companies like Sigma-Aldrich or Thermo Scientific, Micro particles, and Microspheres-Nano spheres offer super paramagnetic beads as well. The primary use of these commercial beads is binding, purification, and magnetic separation of biomolecules comprising proteins, cells, D.N.A. fragments and other biomolecules such as nucleic acids, enzymes, antibodies or bacteria." [13,14]

Experimental Project Hypothesis

In order to verify in clear way the productive strategy in m RNA vaccine manufacturing it is needed to receive from the various producers the complete documentation about the purification methods and the characteristics of the material used. The same this manufacturing process must to be verified by independent professional subjects even if Regulatory GMP verifies was already performed (double check). In this commission a representative of safety for patient organization must to be included to testify the operations. Official documents and photo must to be collected as well as the laboratory chemo analysis related. Impurity (graphene derivate in specific way) using a classic chemico analytical methods whit pre-treatment of the sample (solvent) [15,16]. It is not acceptable that in official report of assessment it is written that it is needed to complete the information about productive production of an innovative m RNA COVID-19 VACCINE as well as the quality certification of the raw material used.

Timing of the verify: at the start of the production and every 6 month the first year, then 1 time every year

The results of this verify must to be of public availability and uploaded officially in producers website.

Discussion

Related the literature reported it is clear that nowadays new methods for purifying RNA are in use versus the classic methods. Large scale production is different vs lab scale. Between various purifying method: reversed phase ion pair anion exchange and affinity chromatography is used and with magnetic beads. Graphene modified magnetic beads show great efficacy in this kind of process. But because m RNA VACCINE manufacturers not clarify in complete way the production process and the toxicological properties of graphene derivate are well knower it is crucial to investigate if this new efficient technology is or not used in today production of some covid-19 vaccine. And related (19 February 2021) EMA/707383/2020 Corr.1*1 Committee for Medicinal Products for Human Use (CHMP) assessment report comirnaty.

"Manufacturers: The active substance is manufactured and controlled by either Wyeth Biopharma Division, Andover, United States or by Biotech Manufacturing GmbH, Mainz, Germany, and Rentschler Biopharma SE, Laupheim, Germany.

During the procedure, a number of issues were highlighted relating to the GMP status of the manufacture of the active substance and of the testing sites of the finished product for the purpose of batch release. These issues were classified as a Major Objection (MO). After further information was obtained from the sites and inspectors, the MO was considered resolved. EU GMP certificates for the manufacturing and testing sites were subsequently obtained. In conclusion, appropriate manufacturing authorizations and GMP certificates are in place for all active substance and finished product manufacturing sites. 2 active substance processes have been used during the development; Process 1 and 2. The major changes between AS Process 1 and 2 are: increased process scale, D.N.A. template changed from a PCR template to linearized plasmid D.N.A., magnetic bead purification replaced with proteinase K digestion and UFDF steps. As regards SO4, the data are requested to be provided regarding the synthetic process and control strategy for the excipient ALC-0315 in order to improve the impurity control strategy, assure comprehensive quality control and batch-to-batch consistency throughout the lifecycle of the finished product:

a) A detailed description of the chemical synthesis of ALC-0315 (e.g. information on reagents and process conditions) should be provided. Due date: January 2021

b) Differences in the manufacturing -process between two suppliers should be described and possible impact on impurity profile should be discussed by July 2021. January 2021

c) Information and justification of quality control of starting materials (e.g. general synthetic route, supplier and specifications) and solvents should be provided. Due date: July 2021, Interim report: January 2021

d) Information and justification on critical steps and intermediates (including specifications) should be provided. Due date: July 2021, Interim report: January 2021

e) Specified impurities should be further evaluated and appropriate specification limits for individual impurities should be included when more data are available. Acceptance criteria for specified and un-specified impurities should be added to the specification for ALC-0315 and should also be evaluated during stability studies. Due date: July 2021, Interim report:

April 2021

f) The specification limit for total impurities should be re-evaluated as more batch data becomes available and revised, as appropriate. Due date: July 2021

g) The specification limit for assay should be tightened based on the provided batch data to improve the quality control strategy of the finished product. Due date: July 2021

h) Detailed method validation reports for assay, impurities, and residual solvents for ALC-0315 should be provided. Due date: July 2021

i) Results of stability studies in accordance with ICH guidelines should be provided. Due date: July 2021, Interim report: April 2021."

Conclusion

It is clear that today m RNA VACCINE manufacturing process use non classic methods in purification phases. Between the more recent technologies are also used affinity chromatography and high-gradient magnetic separation (H.G.M.S.). Magnetic beads are used in this process. Magnetic beads are produced bay various industry using different technology (13). Some producers provide also graphene modified magnetic beads to increase efficiency of the process. For this reason it is necessary that the vaccine producers provide complete and full information about the complete manufacturing process as well as the methodology used in purification. All this related the various technologies in use, the need to get great efficiency in productions and the toxicological implications if impurity of graphene is finded (or not) in final products of vaccine. In natural way it is possible that producers like to use the really best efficient technologies to get better results in manufacturing but this must to be linked to the toxicological limits if dangerous substantive are used in the purification process.

Conflict of Interest

No.

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