




Review Article

Monoclonal Antibodies from Space: Improved Crystallization Under Microgravity During Manufacturing in Orbit



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Abstract

This article explores the significant improvements in manufacturing of monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) enabled by the microgravity environment in orbiting space vehicles. mAbs, which are extensively incorporated into modern cancer treatments based on their ability to specifically target and kill tumor cells, traditionally require intravenous (IV) delivery. However, the inconvenience, potential risks of infection, and adverse systemic effects associated with IV administration have led to a move towards subcutaneous (SC) self-administration formulations. Current limitations hindering the development of SC injections are high viscosity and limited solubility of mAbs at high concentrations. The microgravity environment of space provides potential solutions to these challenges by promoting the formation of colloidal crystalline protein suspensions of low-viscosity and high concentration suitable for SC injection. Although conducting research and manufacturing in microgravity poses its own set of challenges, the benefits of improving the delivery, storage, and stability of mAbs are substantial. SpacePharma has developed novel, autonomous, remote-controlled, microfluidics-based lab-on-chip microgravity systems as a platform for the rapid screening and improved growth of crystallized monoclonal antibodies inside micron-size droplets. The advancements in this field have significant potential to improve patient care by enabling large-scale manufacturing of crystallized mAb therapies in the emerging space economy.

Introduction

Monoclonal Antibodies (mAbs) and their derivatives, such as antibody-drug conjugates (ADCs), are biotherapeutic proteins extensively incorporated into many modern cancer treatments.^{1–3} These proteins specifically target and kill tumor cells, thereby sparing healthy cells. Traditionally, protein-based drugs are primarily ad-

ministered by intravenous (IV) infusion in a hospital/clinic setting, using permanent ports underneath the skin. Given their superficial location on the skin, peripheral veins provide easy access to the circulatory system and are often utilized in the parenteral administration of medications. This treatment regimen typically requires repeated doses at 3-week intervals to maintain efficacious drug concentrations in the blood. Often, these treatments span several months, sometimes even years.⁴

Recently, the practice of administering mAb-based biopharmaceutical treatments has pivoted towards subcutaneous (SC) self-administration, accompanied by improved patient convenience and compliance.^{5,6} Nevertheless, the restricted dosing volume of routine SC injections (up to 2 mL), coupled with the continuously increasing dose requirements of many mAbs and mAb-related products, pose notable challenges in product development, particularly regarding viscosity and stability. The propensity of most mAbs for limited solubility and their substantial increase in viscosity above 100 mg/mL complicate the pharmaceutical development of SC formulations.⁷ Yet, there is a growing unmet medical need for the development of highly concentrated mAb formulations for SC administration.⁸ SC injection could potentially allow for less frequent dosing at the hospital or local doctor's office. Nonetheless, designing a typical therapeutic mAb dose, which often falls in the 150–200 mg range, for SC injection, remains a significant

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Abbreviations: ADC, antibody-drug conjugates; API, active pharmaceutical ingredient; ASI, Italian Space Agency; CAGR, compound annual growth rate; CRS-28, commercial resupply services 28; EMA, European Medicines Agency; ESA, European Space Agency; FDA, Food and Drug Administration; ISA, Israel Space Agency; ISS, International Space Station; IV, intravenous; JAXA, Japan Aerospace Exploration Agency; LOC, lab-on-chip; mAbs, monoclonal antibodies; MARS, Mid-Atlantic regional spaceport; MoTi, modern times module; MSD, Merck Sharp & Dohme; NASA, National Aeronautics and Space Administration; NG-19, Northrop Grumman 19; PEG, Polyethylene Glycol; SC, subcutaneous; SPAd, SpacePharma Advanced Lab; SRV, suborbital reusable vehicle.

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challenge.⁹

Reports in the literature over the last three decades indicate that the microgravity environment can be used to create novel protein crystalline polymorphs with physicochemical properties significantly different from protein crystals grown on Earth at 1g (one g is the force per unit mass due to gravity at the Earth's surface; this standard gravity is defined as 9.8 meters per second squared), thus enabling the production of low-viscosity, highly concentrated colloidal protein crystal suspensions.¹⁰⁻¹² These physicochemical characteristics are essential for developing therapeutic mAb and ADC formulations that are suitable for simple SC injection. These high-concentration crystalline mAb formulations could then ideally be administered at a regular doctor's office or even by patients themselves as home therapy, thereby avoiding the traditional, hospital-based, day-long, IV infusion treatment protocols.¹³

Similarly, the microgravity environment facilitates the formation of high-quality, large, pure, uniform mAb crystals with enhanced physicochemical features and improved characteristics for X-ray diffraction analysis.¹⁴⁻¹⁶ The knowledge gained based on these crystalline protein forms will help in better understanding the functional aspects of monoclonal antibodies, such as epitope binding, and advance the design of new, improved monoclonal antibodies. In view of the biopharmaceutical relevance of therapeutic mAbs manufacturing in space, the objective of this article is to assess methods employing the microgravity environment for the rapid screening and growth of crystallized mAbs, thereby facilitating the development and manufacturing in orbit of novel colloidal crystalline suspensions suitable for SC administration.

Monoclonal antibodies: leading biotherapeutics, market growth, and development challenges

Since the Food and Drug Administration's (FDA) approval of Orthoclone OKT3 (muromonab-CD3), the first monoclonal antibody approved for therapeutic use in 1986,¹⁷ biologics such as mAbs and ADCs account for nearly a fifth of the FDA new drug approvals each year.¹⁸ With nearly 140 approvals by the FDA and European Medicines Agency (EMA) in total, these therapeutic antibodies have revolutionized the treatment of diverse diseases such as cancer, autoimmune disorders, infectious diseases and inflammatory conditions. They are a preferred treatment modality due to their high specificity, potential for improved patient outcomes and reduced side effects profile compared to traditional therapies.¹⁹

The market for therapeutic antibodies has witnessed significant growth in recent years and is projected to continue expanding. The increasing prevalence of chronic diseases, advancements in antibody engineering technologies and the growing demand for targeted and personalized therapies are driving the market's growth. The global market for therapeutic antibodies was valued at USD 200 billion in 2022 and is expected to reach USD 327 billion by 2027, with a compound annual growth rate (CAGR) of 8.9% during the forecast period.^{20,21} Consequently, pharmaceutical companies and biotechnology firms are heavily investing in antibody research and development.

Nonetheless, this field faces challenges regarding manufacturing, purification, formulation, delivery, and storage of mAbs. The purification process, in particular, is a major bottleneck in mAb production, constituting up to 80% of the manufacturing costs.^{22,23} Crystallization is a potential solution to reduce these costs. Although successfully applied to small-molecule drugs and peptides, crystallization processes applied to larger proteins like mAbs have primarily focused on structural determination through X-ray crys-

tallography, with limited consideration for large-scale manufacturing and downstream processing.

At present, mAb preparations have a limited shelf life and require refrigerated storage conditions. They are stored as drug substances in large, impractical bags until formulation into small dosage forms which also necessitate refrigerated storage. The development of high-concentrated, non-viscous mAb formulations that are stable at room temperature would be highly beneficial. Such innovation would allow these biologics to be transported in smaller containers to production sites globally, thereby addressing the existing storage and transport challenges in many parts of the developing world.

Subcutaneous injection versus intravenous administration of mAbs

mAbs are primarily designed for IV administration. Among the 35 new FDA drug approvals for 2022, nine (26%) were mAbs (~70% of all the biologic drugs approved) and all of them are indicated for IV infusion.²⁴ The standard process of administering therapeutic antibodies via IV infusion, however, presents logistical challenges and requires significant resources. Patients often face long-distance travel for hospital care, increasing inconvenience and infection risks. Furthermore, patients with vascular conditions may require surgically implanted IV ports, further complicating the treatment process. In contrast, SC administration of mAbs and other therapeutic proteins offers a promising alternative due to its less invasive nature, shorter administration time, and improved patient convenience. This approach eliminates the need for repeated IV injections as well as long-term central venous access devices and it reduces patient discomfort associated with infusion-related reactions and potential infections. SC injection is safer and more convenient, particularly for patients with poor venous access.²⁵ The market trend also reflects a preference for SC delivery of biologics, with an increasing number of mAbs being developed for SC administration.²⁶ This trend is supported by the improved efficacy and safety profiles of SC mAbs delivery, which is well-documented across a range of therapeutic areas.^{19,27}

Despite this positive outlook, the use of SC administration for mAb parenteral drugs has been limited, primarily because SC dosing is typically restricted to small volumes that do not exceed 2 mL due to tissue backpressure. This backpressure can result in drug loss due to seepage and increased injection pain. Nevertheless, from a patient's perspective, the SC route is less invasive than the IV route, making it highly preferable for self-administration in a home-care setting. The demand for autonomous self-administration of SC delivered therapeutic mAbs is therefore continuously increasing.²⁸ Despite this, SC mAb formulations do pose challenges, such as high viscosity, opalescence, aggregation, and potentially detrimental pharmacokinetic effects. To address these issues, numerous formulation development approaches have been explored, including crystallization, enzymatic depolymerization, nanosuspensions, molecular design, glycoengineering, and nanobodies.^{8,26,29}

The crystallization of mAbs to form colloidal suspensions of crystalline particles provides an alternative solution for SC administration of high mAb doses. Colloidal crystalline formulations typically have lower viscosity compared to similar concentrated solution formulations. For instance, crystalline suspensions of mAbs, i.e. Infliximab, at 150 mg/mL exhibit acceptable viscosity values (26 cP) compared to the unacceptable viscosity (275 cP)

observed in comparable solution concentrations. In addition, the pharmacokinetic profile of such SC crystalline mAbs has been shown to be remarkably long, something that may prove beneficial in certain disease treatment approaches.²⁶ Furthermore, crystallization can enhance the stability, shelf-life and storage of colloidal nanosuspensions of mAbs for extended periods of time. Such formulation of mAbs could potentially eliminate the need for refrigerated storage and transportation completely, thus lowering patient costs and enabling distribution of such drugs in areas of the world that lack refrigeration storage.³⁰ While crystallized mAbs for SC delivery do seem like a promising and novel therapeutic approach, more research is warranted. Initial studies on safety are positive, though knowledge and consensus on the immunogenicity of SC mAbs in crystal form is still lacking.³¹

Protein crystallization methods

A variety of methods to produce crystals of proteins are currently available, including the vapor diffusion, dialysis, and batch methods.³² Vapor diffusion, performed either by the "hanging-drop" or "sitting-drop" technique,³³ is the most commonly used method today. Vapor diffusion involves equilibration of a droplet containing a mixture of protein and precipitant solution against a reservoir of pure precipitant. As equilibration proceeds, the concentration of protein and precipitant in the drop slowly increases until the correct concentration for crystallization is achieved.³⁴ The dialysis method is based on slow diffusion of a protein solution against a precipitant solution through a semi-permeable membrane that allows only small molecules and ions to pass, leading to crystal formation once the correct solute concentration for crystallization is established.³⁰

The batch method for crystallization, in contrast, is not based on diffusion, and includes direct mixing of the protein solution with a precipitant solution at a pre-set supersaturated concentration, resulting in the direct initiation of protein crystal formation.³⁵ A variation on this method is the micro-batch technique, where small volumes of protein and super-saturated precipitant, as little as 0.5 μ L, are dispensed under oil to minimize evaporation.³⁶ The advantages of this method are minimal protein consumption and crystals that are more stable when compared to the diffusion-based methods due to constant conditions, constant temperature, and protection from contamination.^{37–40} In addition, it is a highly suitable method for automated crystallization workflows.^{41–44}

Despite the numerous methods available today for crystallization, challenges persist in the generation of protein crystals of sufficient quality for data collection and structural determination using X-ray diffraction. Crystal production is hampered by the dynamic, flexible, and sensitive nature of proteins, which leads to difficulties in obtaining a uniform and ordered protein crystal lattice. In addition, most protein crystals have few intermolecular contacts, high solvent content, high density of defects, and high sensitivity to radiation and changes in temperature.^{45,46} Considering these issues, obtaining protein crystals of sufficient quality is greatly dependent on the purity of the sample, the conformational homogeneity of the protein, and the absence of denatured molecules and micro-aggregates. Crystal quality is also significantly affected by physicochemical processes that occur during crystallization, specifically mass transfer. Mass transfer occurs via two mechanisms: diffusion and gravitational convective flows. The latter cause instability and fluctuations that lead to aggregation and clusters in the crystallization protein solution, generating defects and affecting crystal growth and crystal perfection.⁴⁷

Microgravity environment

Microgravity serves as a unique disruptive environment that accelerates biological and chemical systems by enabling rapid 3D growth of bacteria and viruses as well as improved crystallization of proteins, antibodies and antiviral agents.^{48–50} Microgravity thus facilitates the discovery of new drug candidates by accelerating the screening process and identifying new drug-protein receptor drug/target interactions through enhanced co-crystallization of antibodies and antiviral/protein targets.⁵¹ At the molecular and cellular level, the microgravity of space can alter the physicochemical properties of protein crystals, membranes and cells. Such alterations may be harnessed for better understanding of the structure of blood-brain-barrier receptors and therapeutic proteins.⁵²

An important aspect of the impact of microgravity is that it substantially improves the growth of protein crystals.⁵³ Reduced-gravity conditions may improve crystal formation for proteins that are prone to forming disordered aggregates at high concentrations, such as huntingtin, due to a reduction in buoyancy-driven convection.⁵⁴ In low-convection environments such as in microgravity, mass transport and movement of protein molecules is primarily driven by random diffusion and is therefore much slower than on Earth. This results in aggregates diffusing more slowly than monomers and therefore in microgravity monomers may have greater access to the surface of the growing crystal than aggregates.⁵¹ In addition, sedimentation effects caused by gravitation are absent, and there is no pressure on the crystals caused by contact between the crystals and the surface of the vessel containing them. Taken together these factors lead to reduced movement of the crystal with respect to the surrounding fluid, resulting in a more steady, ordered, and isotropic crystal growth, which ultimately produces larger and less mosaic crystals with better optical properties, higher purity and higher diffraction power.^{55–60} Comparison of structural data from space-grown and Earth-grown crystals indicates that microgravity does not alter the native conformation of protein chains, and confirms unequivocally that space-grown crystals provide 3D-structures of superior definition, order and accuracy.⁵⁵ Similarly, when gravity is eliminated as a masking factor, other interactions can become more prevalent. This can lead to crystalline structures (polymorphs) that are unique compared to crystals formed on Earth. Such an environment may potentially facilitate the crystallization of materials which could not be successfully crystallized under normal gravity conditions at all.^{53,61}

Following crystallization in space, the resulting crystals can be returned to Earth for subsequent protein mapping, X-ray diffraction and crystallography analysis. This approach has been applied to hematopoietic prostaglandin D synthase, a protein expressed in certain muscle fibers of patients with muscular dystrophy. Crystallization of this protein in space resulted in the discovery of a new inhibitor, found to be several hundred times more potent than the original drug.⁶² A major contributor to this discovery has been the Japan Aerospace Exploration Agency (JAXA), which has been actively involved in conducting protein crystallization experiments since the 1990s. They have concentrated their efforts on improving protein sample conditions and developing crystallization containers specifically designed for space experiments, aiming to maximize experimental results.⁶³

Nevertheless, performing crystallization experiments in space is not simple, and a variety of new issues must be addressed for it to be successful. Many technologies and devices for space crystallization have been developed and implemented to cope with these problems, either to be activated automatically when reaching zero-gravity or activated with minimal participation of the crew.⁶⁴

One of these developments includes a Vapor Diffusion Apparatus (VDA) containing an array of cells, with two syringes that can form a crystallization drop from protein and precipitating agent solutions after reaching zero gravity. Before landing, the piston can move in the reverse direction to draw the drop with grown crystals into the syringe for protection.⁶⁵ Another method, implemented in the Mir space-station, involves freezing the protein and precipitating solutions in liquid nitrogen prior to space shuttle launch, thereby enabling synchronization of crystal growth by diffusion with entrance into orbit, following a slow evaporation of nitrogen from the Dewar (a cryogenic storage vacuum flask used for storing cryogenics, such as liquid nitrogen, whose boiling points are much lower than room temperature).⁶⁶ More recently, the Granada Crystallization Box (GCB) and the JAXS Crystallization Box (JCB) have been also introduced.^{67,68} These boxes are based on the capillary counter-diffusion method for crystallization⁶⁹ and contain capillaries with the protein and precipitant solutions separated by a gel layer, allowing crystals to form stably while counter-diffusing through the gel.⁶⁸

The return of the produced crystals from space to earth also poses certain challenges regarding preserving the relatively fragile protein crystals. While precise temperature control and refrigeration for increased stability is often a possibility, mechanical stresses upon spacecraft re-entry are harder to eliminate and may pose a risk to the stability of the produced crystals. Nevertheless, the numerous examples of protein crystals being returned to earth successfully show that this risk is not significant.⁷⁰

Microgravity and mAb crystallization

The favorable properties of microgravity for crystallization expedite the growth of high-quality, large, pure, and uniform mAb crystals with improved physicochemical properties. Crystallizing mAbs in microgravity can potentially enable their delivery in more concentrated doses via SC injections, hence offering a method for improving both drug formulation and storage.¹⁷ A quintessential example of successful research in microgravity involved the growth of highly ordered, uniform crystalline particles of the therapeutic mAb Pembrolizumab (Keytruda®, Merck & Co.), leading to improved drug stability and storage.¹¹ Although the three-dimensional structure of Pembrolizumab has been determined through *X*-ray crystallography, the high salt crystallization conditions used in the process were deemed unsuitable for formulation and delivery applications. Consequently, polyethylene glycol (PEG)-based crystallization conditions suitable for drug delivery applications were identified, but further optimization was required to establish an efficient batch crystallization process. This pioneering work served as proof of concept, demonstrating that a microgravity crystallization experiment involving a full-length mAb could produce a homogeneous crystalline suspension with reduced viscosity and improved rheological properties, as opposed to the bimodal crystalline suspension observed in Earth-based control experiments.

Although microgravity research comes with its own set of challenges, including adapting Earth-based processes to flight-certified hardware, dealing with experimental timelines, and limited real-time analysis capabilities, microgravity research offers unique opportunities to generate materials that differ from those produced in traditional Earth-based experiments, potentially leading to results that can reshape the design and planning of experiments and processes on Earth.

There are several relevant publications by NASA and the sci-

entific community referring to work performed by pharmaceutical companies on the improved crystallization, growth, and production of crystalline monoclonal antibodies for pharmaceutical applications in microgravity, as described by Braddock.⁷¹ In this article Braddock lists several biopharmaceutical companies that have attempted to derive protein structures from *X*-ray crystallography of crystals grown in microgravity. The list includes several selected drug/target proteins or mAb therapies such as Keytruda (Merck & Co.),¹¹ reverse transcriptase (Wellcome),⁷² interferon alfa (Schering-Plough),⁷³ and membrane protein transcriptase (Lilly).⁷¹

Merck & Co. (MSD) is also attempting to improve the purification process involved in manufacturing therapeutic monoclonal antibodies. Currently, monoclonal antibodies are produced using cell culture fermentation, followed by multiple steps to purify and isolate the active pharmaceutical ingredient (API) from the fermentation broth using chromatography techniques. This purification process is effective, but it is also very time consuming and expensive. MSD is testing whether crystallization could be used to purify monoclonal antibodies directly from the fermentation broth, thus significantly reducing or eliminating the need for extensive chromatography and, in turn, lowering production costs.¹¹ Bristol Myers Squibb (BMS) is also currently active in space, investigating the structural and crystallization kinetics of monoclonal antibodies on board the International Space Station (ISS).⁷⁴ Additionally, AstraZeneca, in collaboration with MedImmune, is investigating the stability of mAbs in a microgravity-formulation study.⁷⁵ Storing formulations in microgravity may reveal processes that lead to degradation and, ultimately, to methods for deterring them.

Enabling technology for mAb crystallization in microgravity

Despite the development of space crystallization technologies in recent years, it has become apparent that most methods are not sufficiently precise, sufficiently automated, and sufficiently stable. In addition, many of these methodologies require relatively large volumes and use relatively large amounts of protein. In addition, the retrieval and harvesting of the resulting space-crystals is still an issue in most of these technologies. To address these shortcomings, the company SpacePharma has developed a novel microfluidics lab-on-chip (LOC) device based on the microbatch crystallization method. This implementation allows for the continuous and efficient crystallization of proteins in space and provides the capability to test multiple crystallization parameters within a single mission in order to pinpoint optimal conditions for obtaining large, superior quality crystals. These LOCs are housed in and operated through the SpacePharma Advanced Lab (SPAd), (Fig. 1) which consists of fluid reservoirs, a dispensing fluid unit, a manifold which directs fluid flow, a x/y/z scanning system for scanning the LOCs using a miniature microscope (visible light and fluorescence) and spectrometer (wavelength range of 420–750 nm), a service unit, refrigerator, syringe pumps, and an on-board computer. The patented fluid handling system, composed of active and passive pumps and reservoirs for the solutions and reagents, is modular and connected to each LOC through a “plug and play” module.^{76–78}

The SPAd lab can contain up to 3 LOC devices and provide all interfaces (flow, mechanical and electrical) to the LOCs, including temperature control (4–37°C) (Fig. 1a). Active syringe pumps (up to 4) connected through a manifold to passive pumps (up to 24) produce controlled flow of reagents to specific LOC ports with flow rates in the range of 10–500 µL/min. In addition, the passive pumps can draw reagents from the 4–25°C refrigerator unit and

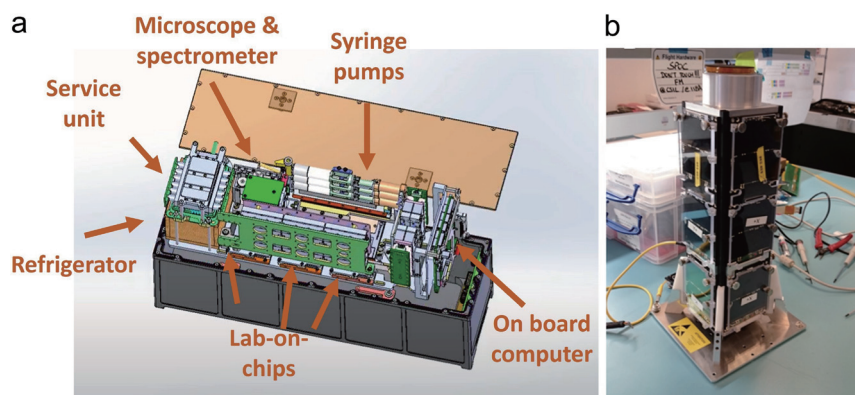


Fig. 1. SpacePharma's microgravity research platforms. (a) Schematic illustration of SpacePharma Advanced (SPAd) lab including the scanning system, microscope, spectrometer, lab-on-chips (LOC), service unit, refrigerator, syringe pumps, and on-board computer. (b) SPAd in SpacePharma's final assembled DIDO nanosatellite.

deliver them accurately to specific LOC ports. The product or sample flows out of the LOC into a collection bag kept in the 4–25°C refrigerator or into a 1 mm diameter collection tube for fractional collection. The SPAd service unit enables filling the LOCs and reservoirs with reagents after the assembly of the SPAd-lab. The entire system is located inside a pressurized atmospheric box, which can be housed in the ISS as-is, or be placed inside of the DIDO nanosatellite (Fig. 1b).⁷⁸ The DIDO-3 nanosatellite consists of three CubeSat units, one of which for the service module and two for the actual SPAd microgravity laboratory with dimensions of 35 × 10 × 10 cm and a total weight of 5.6 kg. The service module comprises an on-board computer and a battery package.⁷⁹ The DIDO nanosatellites orbit at an altitude of 500 km and are equipped with solar cells and a communication system that can be remotely controlled through a simple application.

The remote-controlled SPAd labs have a modular design that is adaptable to meet the specific needs of researchers. The labs are simplified, accessible, affordable, end-to-end microgravity solutions that can be controlled in near real-time via a web-based interface. This innovative approach to space-based drug research and crystallization experiments increases accessibility to the unique environment of microgravity, in part also by eliminating the need for astronaut intervention. In 2018, SpacePharma verified the effectiveness of their developments by employing the miniaturized microgravity lab to execute successful crystallization experiments

during a NoveSpace/Zero G parabolic flight mission. The resultant crystals displayed enhanced purity, uniformity, and size when compared to 1g ground control. It was demonstrated that the creation of small quantities of seed crystals in microgravity was sufficient for seeding similar polymorphs on Earth, facilitating lower resource requirements in the process.⁸⁰ Since this pivotal experiment, the company has demonstrated the efficacy of its microgravity-research enabling technology through 9 launches to space, comprising 2 autonomous nanosatellites and 7 round trips to the International Space Station (ISS). SpacePharma's microgravity-based lab platforms and crystallization processes are protected by several assigned patents and recognized by space agencies globally, including NASA, European Space Agency (ESA), Italian Space Agency (ASI), and Israel Space Agency (ISA).^{48,81–85}

On August 2nd 2023 SpacePharma launched its first mission to crystallize a mAb in orbit using the SPAd microgravity lab (Fig. 2) on the Northrop Grumman 19th mission (NG-19) Cygnus spacecraft aboard the Antares rocket from the Mid-Atlantic Regional Spaceport (MARS) on Wallops Island, Virginia.⁸⁶ After connection of the system to the power supply of the ISS and activation, the therapeutic mAb solution was mixed with an equal amount of precipitant antisolvent (composed of low and high molecular weight polyethyleneglycols (PEG) and ammonium sulfate 3.5 M solution) to induce crystallization. The experiment was returned to Earth on September 2nd 2023 aboard the commercial resupply ser-

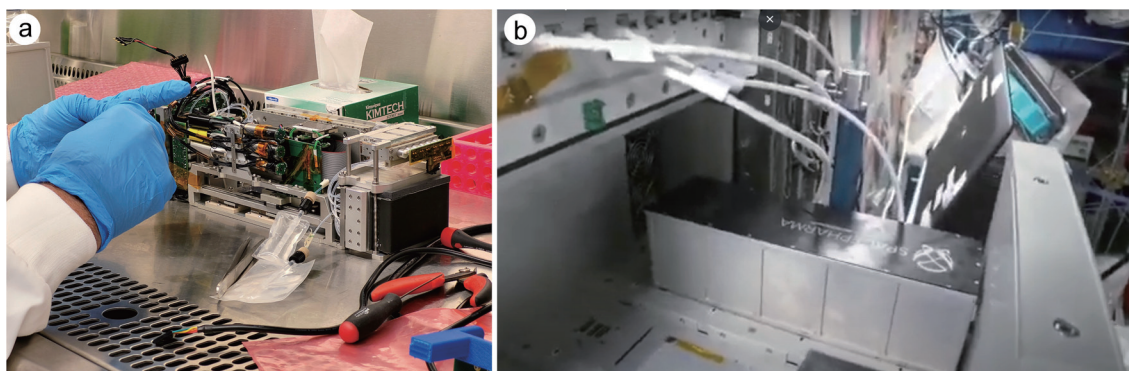


Fig. 2. SPAd microgravity lab assembly and operation. (a) the SPAd being assembled and filled with reagents in a sterile laminar hood before launching into orbit on a Northrop Grumman 19 (NG-19) Cygnus spacecraft aboard the Antares rocket. The visible plastic bags are part of the reservoirs containing mAb solutions, antisolvent and reagents. (b) SPAd microgravity lab installed in the International Space Station (ISS) (Courtesy of NASA).

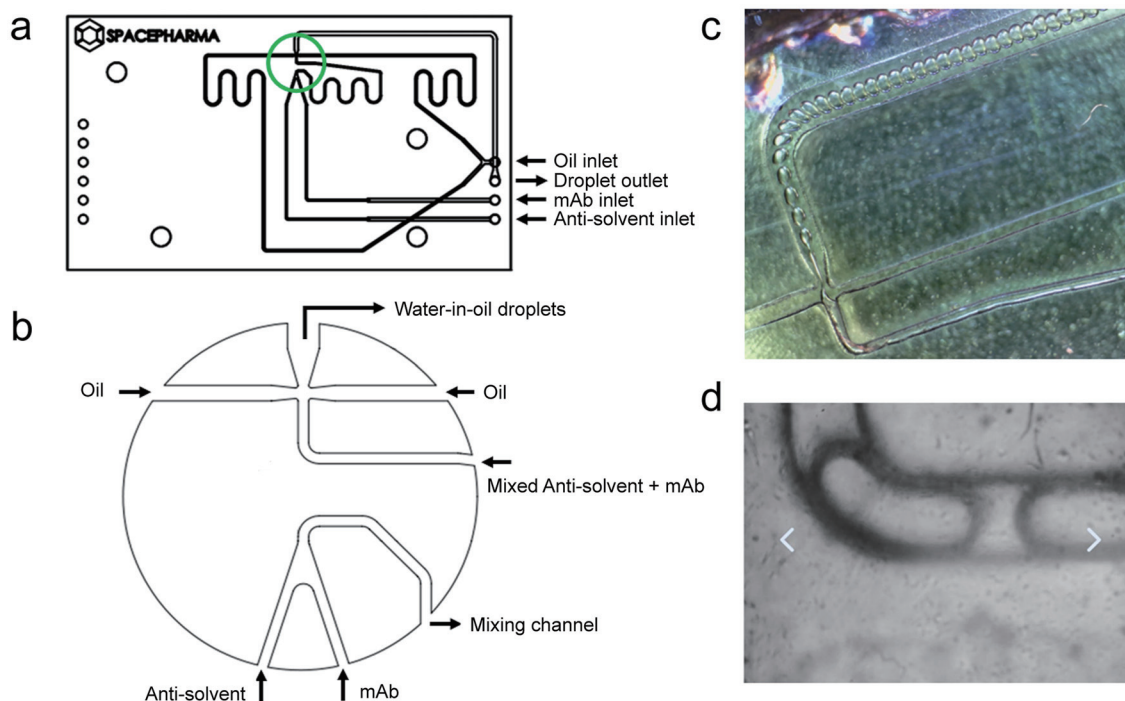


Fig. 3. Schematic configuration of customized lab-on-chip design for mAb crystallization in orbit. (a) Microfluidic chip where monoclonal antibody (mAb) dissolved in buffer, precipitant antisolvent solution and oil are injected into the chip through different ports. (b) Close-up of the microfluidic junctions circled green in A. The dissolved mAb and antisolvent meet at a junction and are transported to a mixing channel, after which this flow is cut by two oil flows, creating water-in-oil microdroplets. (c) Water-in-oil microdroplets right after generation. (d) In orbit, water-in-oil microdroplets containing mAb and antisolvent as visible from space through the SPAD's onboard light microscope.

VICES mission 28 (CRS-28) SpaceX dragon spacecraft for analysis and subsequent comparison to Earth-based controls.

Droplet microfluidics technology for mAb crystallization in space

Droplet-based microfluidics is a valuable tool for experiments involving high-throughput protein crystallization screening. Since each droplet is viewed as a separate microreactor, a large number of experiments can be conducted under the same conditions, providing greater versatility with minimum material use.⁸⁷ Additionally, the depth of the crystallization chamber can also be used to control the shape of the protein crystal.⁸⁸ Droplet-based microfluidic systems enable the generation of homogeneous, uniform and stable droplets with a superior frequency allowing more flexibility with regard to the produced droplet volume.⁸⁹ SpacePharma's LOC systems employ droplet microfluidics technology to encapsulate and manipulate mAb molecules within micron-sized water-in-oil droplets. This approach enables precise control over the crystallization and formulation processes, allowing for the optimization of antibody concentration, stability and compatibility with SC injection. Using such droplet-generating microfluidics, multiple parameters such as buffer conditions, pH, and excipients can be efficiently tested. The controlled particle size distribution and stable colloidal crystalline suspension properties achieved through microgravity-based crystallization can potentially facilitate efficient drug delivery and effectiveness of SC injection, leading to improved medical treatment outcomes.

An example of SpacePharma's customized LOCs for mAb crys-

tallization is depicted in Figure 3a. This small LOC of $40 \times 70 \times 4$ mm (W \times L \times H) in size contains a fluid handling system that can be monitored by a miniature light microscope and spectrophotometer. The light microscope includes a camera that captures and transfers images to a proprietary web system for controlling experiments and viewing their results. The chips are rigid, robust and can endure the different challenges of space missions, such as vibrations at the launch take off and during the return to Earth, thus enabling an extended stay in space.

The LOC contains microfluidic channels that combine and mix a mAb solution and antisolvent solution to initialize crystallization. This flow is "cut" from both sides by a controllable oil flow to form oil-in-water microdroplets of 1–10 μ L (Fig. 3b–d). By controlling the flow rate of the different solutions using non-pulsating syringe pumps, microdroplets are generated with different ratios between the mAb, buffer and precipitant solutions. Each microdroplet, containing a different chemical composition, is equivalent to a single micro-batch crystallization condition. The oil surrounding the droplets will prevent any interaction between the microdroplets, and thus each droplet will represent an independent crystallization experiment. To create different ratios between the protein/buffer/precipitant and to adjust the microdroplet size and frequency of the droplet production, the controlled flow rate of the pumps is varied. Since temperature is an important factor in the crystallization process, the temperature of the LOCs is monitored and carefully controlled as well. After droplet generation, the flow is stopped, and the microdroplets are incubated at a desired temperature. Crystal formation is monitored with a light microscope with magnifications ranging from $\times 10$ to $\times 40$. Once crystals are formed, they are removed from the LOCs by gentle ejection with

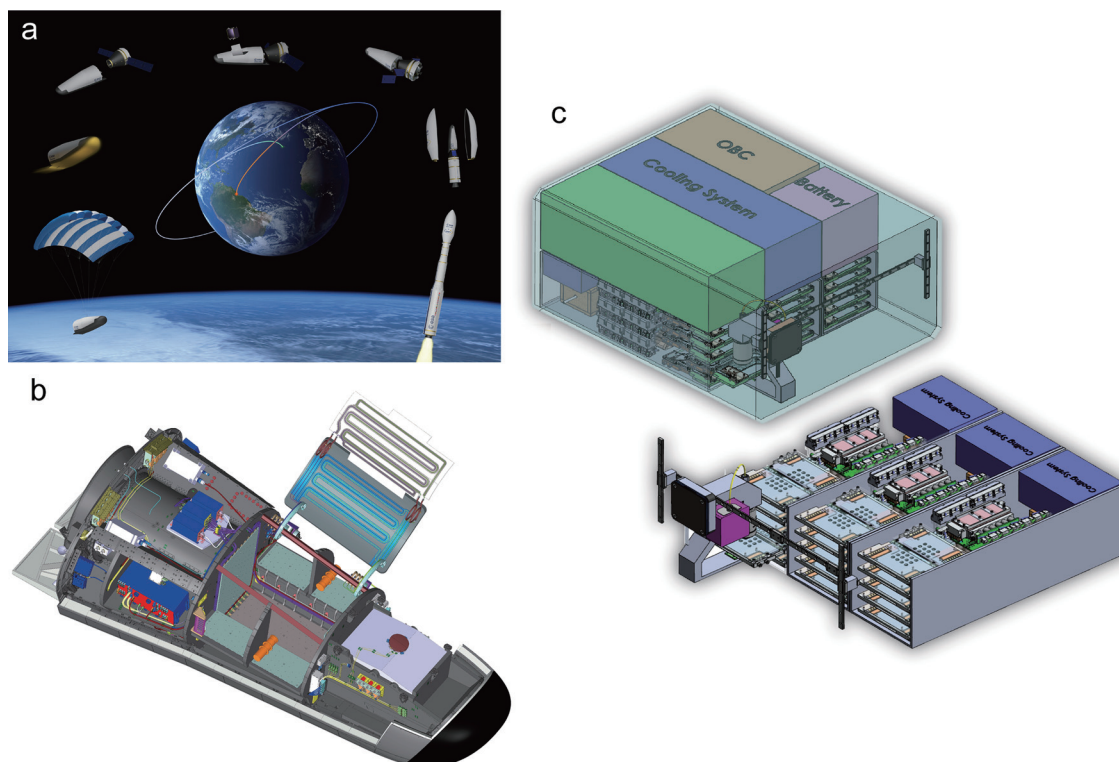


Fig. 4. Future platforms and manufacturing system. (a) Schematic representation of an ESA SpaceRider minishuttle mission; courtesy of European Space Agency (ESA). (b) Illustration of SpaceRider minishuttle reusable space vehicle; courtesy of ESA. (c) SpacePharma's MoTi in-orbit large-scale production platform.

a pump, isolated and mounted for traditional X-ray diffraction data collection with cryo-protectants. Alternatively, the LOCs could be mounted directly on a dedicated 4-circle goniometer (a device that measures an angle or permits the rotation of an object to a definite position) and subjected to serial *in situ* X-ray diffraction studies at room temperature or flash-freezing conditions.^{53,59,60}

Suborbital reusable mini-shuttles and manufacturing in orbit

Suborbital Reusable Vehicles (SRVs) or small shuttle-like orbital vehicles are uncrewed spacecrafts which are becoming utilized as an alternative to the ISS to address the increasing demand for scientific experimentation and manufacturing in space. They offer opportunities for conducting more affordable science missions in space, with more frequent flights and shorter waiting times for experiments than the currently used crewed platforms.⁹⁰ SRVs such as Starship (SpaceX), Dream Chaser (Sierra Nevada), X-37B Orbital Test Vehicle (Boeing), Space Usable Dragon (AXIOM) and Space Rider (ESA), are being developed by private companies and national space agencies. These platforms are creating a new space-flight industry that will foster larger-scale biomedical experiments and manufacturing in space with the ability of returning their payloads to Earth, an infrastructure that is crucial for large scale mAb crystallization in space.

SpacePharma has been certified as a sub-aggregate service provider for ESA's Space Rider mini-shuttle flights with a reserved 100 kg payload in the form of SpacePharma's Modern Times (MoTi) module lockers (Fig. 4). The MoTi module is a fully remote-controlled system designed to redefine large-scale production under

microgravity conditions. It consists of four automated microgravity production lines, each equipped with high-throughput experiment and manufacturing capabilities. The total volume of the Cubesats within the module is 24 U (24,000 cm³), allowing for the containment of 40 kg of desired liquids and components. The MoTi module utilizes non-pulsatile syringe pumps developed by SpacePharma and supported by an EU H2020-SME2 grant 718,717.

This cutting-edge platform sets the stage for the crystallization of dozens of kilograms per mission in a microgravity environment, enhancing the reproducibility and effectiveness of the process through continuous production. It employs technologies trialed on the smaller SPAd platform, effectively being a scale-up version of the proven micro-batch protein crystallization approach. The system's versatility also enables integration with various communication and electric platforms found on the International Space Station (ISS) and on other autonomous SRV's such as the Dream Chaser. This integration provides flexibility and independence regarding launch opportunities.

Future directions

The unique nature of microgravity encountered in space provides an opportunity for drug discovery and development that cannot be replicated on Earth. From the production of superior protein crystals to the identification and validation of new drug targets to microarray analyses of transcripts attenuated by microgravity, there are numerous published examples that demonstrate the benefit of exploiting the space environment for pharmaceutical and biopharmaceutical manufacturing. Moreover, studies conducted on Space

Shuttle missions, the ISS and other crafts have had a direct benefit on drug development programs.

Colloidal crystalline protein formulations offer lower viscosity compared to concentrated solution formulations, making them more suitable for SC delivery. However, further research and optimization are required to establish efficient batch crystallization processes that are suitable for cost-effective formulation and delivery applications.

Microgravity research comes with challenges such as adapting Earth-based processes to flight-certified hardware, buffers, media, chemicals, as well as managing experimental timelines, and limited real-time analysis capabilities. Moreover, the development of good manufacturing practices (GMP) needs to be addressed in space. Despite these challenges, microgravity research offers unique opportunities to generate or reformulate biologicals with distinct properties, potentially leading to unexpected results that can reshape experiments and processes on Earth.

The application of microgravity-controlled crystallization processes in mAb manufacturing, delivery, and storage offers significant opportunities for advancement. Crystalline formulations can overcome challenges related to high viscosity, formulation stability, and storage requirements. Through research and optimization in this field, advancements have already been made in formulation stability, cost reduction, and treatment options for patients.

To fully leverage the potential of colloidal crystalline formulations and to advance the SC delivery of mAbs, further research, development and optimization are necessary. This includes exploring novel crystallization techniques, understanding the impact of crystalline formulations on drug efficacy, immunogenicity, and pharmacokinetics, and developing scalable, GMP manufacturing processes. By addressing these challenges, the field can overcome limitations associated with IV infusion, enhance patient care, and improve the delivery and effectiveness of mAb therapies.

Conclusions

MABs and their derivatives, such as ADCs, are essential modalities in current molecularly-guided cancer treatment strategies. Nevertheless, the current IV delivery method poses significant inconveniences to patients, including the frequency of hospital visits and a potential risk of infection. Consequently, a growing trend towards SC self-administration has been observed. While this method improves patient convenience and compliance, it carries substantial challenges including limited solubility and increased viscosity at high concentrations. The microgravity environment provides a unique platform for addressing these challenges, allowing the creation of low-viscosity, highly concentrated colloidal protein crystal suspensions which are better suited for formulation into SC injections. Initial research and development of colloidal crystalline nanosuspensions of mAbs crystallized in orbit show promising improvements in their crystallization patterns and physicochemical properties. This could transform current mAb IV formulations into innovative high-concentration SC formulations that are low in viscosity, stable at room temperature, and possess extended shelf-life. The future of large-scale manufacturing of crystallized mAb therapies in space can be envisioned.

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Conflict of interest

All authors are employees of SpacePharma R&D, Israel.

Author contributions

Contributed to study concept, design, writing and supervision (SA), drafting and critical revision of the manuscript (DK), design, technical support, data acquisition, and assay performance (DK, OL, AL, YF), contributed to research and review of this manuscript (DB).

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