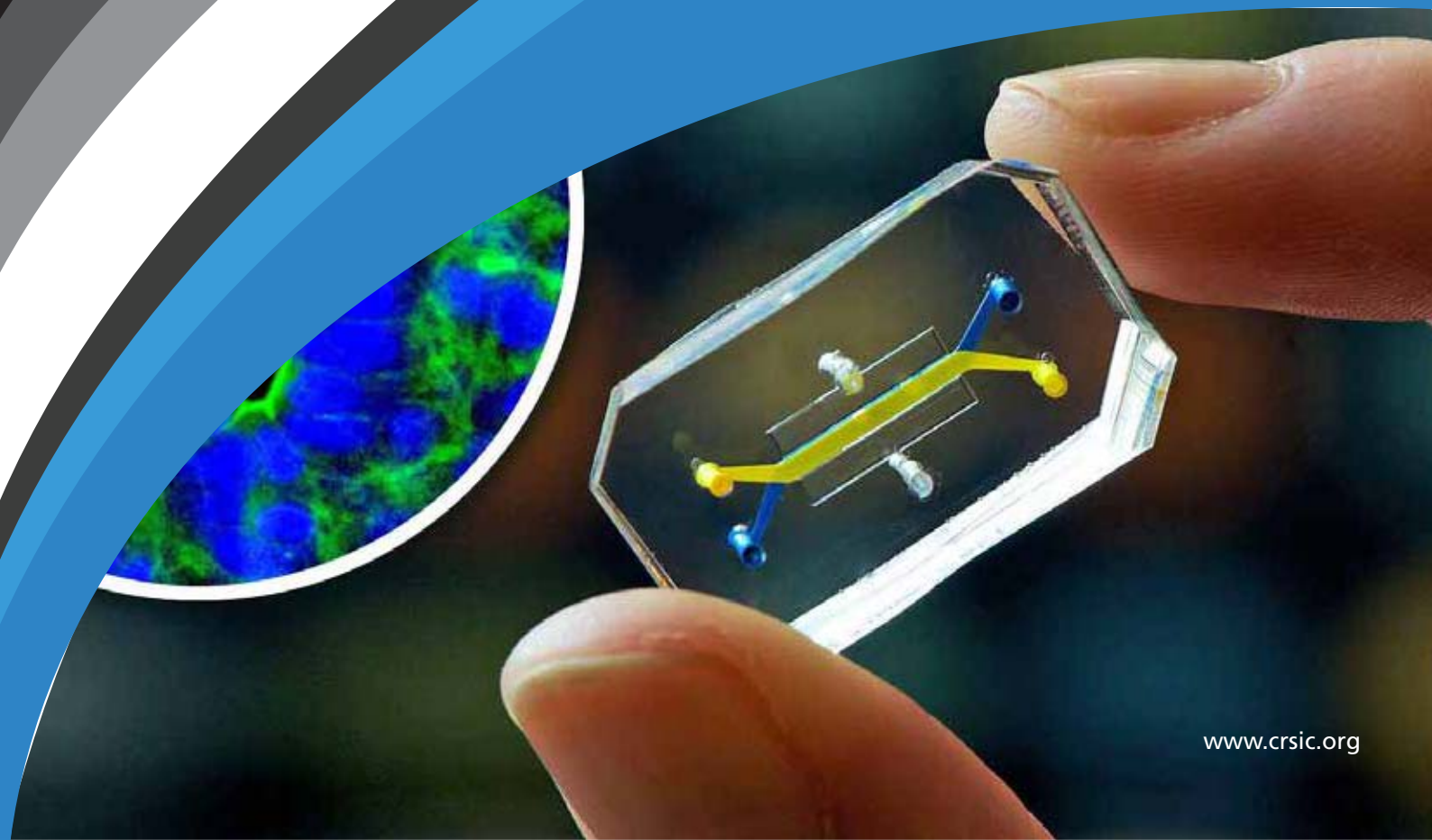
# NEWSLETTER 2021

VOLUME 12

CRS CONTROLLED RELEASE SOCIETY INDIAN LOCAL CHAPTER



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CRS CONTROLLED RELEASE SOCIETY INDIAN LOCAL CHAPTER

# NEWSLETTER

Volume 12, February 2021

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# From the President

**Dr. Parizad Elchidana**
President - CRS Indian Chapter
(2019-2021)

Hi friends, this is my second year in office as the President of Controlled Release Society Indian Chapter (CRS IC), and it is that time of the year when we host our International Symposium. On this occasion of the 19th International Symposium on 'Advances in Technology and Business Potential of New Drug Delivery Systems', which is being held as a virtual event, I am delighted to share with you the 12th edition of CRS IC Newsletter highlighting various diversified technologies in drug delivery and innovative practices.

The pharmaceutical industry is responsible for the research, development, production, and distribution of medications and has been at the forefront of the battle against COVID 19, whether it is development of diagnostic kits or prophylactic vaccines or treatment. Hence the Pharma market has continued to experience significant growth even during the current pandemic times.

The Indian pharmaceutical industry is the world's third largest drug producer by volume and the country's manufacturing units manufacture 60 percent of vaccines globally. We have proved to be the world's largest vaccine manufacturer & supplier at low cost and affordable rates. This talks volumes of our intellect and capabilities.

Side by side, advances are happening globally in the field of biotechnology, nanotechnology, 3D printing, personalised medicine, gene therapy etc., but the importance of patient oriented research continues to play a pivotal role, thus stressing more and more on the importance of drug delivery and translational research.

CRS IC continues to be dedicated to research in drug delivery and supports and promotes scientific innovation nurturing young talent. I urge you all to join the organization and help us expand the base.

I sincerely thank the contributors to this newsletter along with the editorial team, for their persistent effort in launching this noteworthy edition of the CRS IC Newsletter !!

Dr. Parizad Elchidana


PENSAN

# Editor's Note

**Prof. Vandana B. Patravale**
Chief Editor, CRS IC Newsletter

Dear Readers,

I am delighted to welcome you all to the 'Controlled Release Society Indian Chapter' and the Nineteenth International e-Symposium on 'Advances in Technology & Business Potential of New Drug Delivery Systems'. This year marks the inauguration of the twelfth issue of CRS IC Newsletter which aims to enlighten the delegates with interesting articles pertaining to innovative developments in the dynamically evolving pharmaceutical industry.

I am certain that the articles presented in this issue of our newsletter by eminent authors from around the globe will be a captivating read to all. The article by Dr. Kristy Ainslie from the University of North Carolina will talk about the potential of acetalated dextran for enhanced delivery of subunit vaccines. Dr. Ofra Benny from the Hebrew University of Jerusalem sheds some light on the ever-evolving subject of "tumor-on-chip" for personalized medicine.

We have Dr. Natalie Artzi from Brigham and Women's Hospital, Harvard Medical School who will highlight our readers about the delivery strategies and clinical potential of STING (Stimulator of Interferon Genes) agonists in cancer immunotherapy. Followed by this, we have an article contributed by the students of Institute of Chemical Technology, Mumbai on the very interesting aspects of computational pharmaceutics and its changing paradigm. The final article by Dr. Clara Fernandes from Bombay College of Pharmacy, Mumbai gives an update on the applications of ionic liquids in drug delivery. The readers would be exposed to numerous brain teasers throughout the newsletter to make the reading process both exciting and relaxed.

Citing the ongoing global pandemic, the editorial board is highly obliged towards all the contributing authors for sparing time from their demanding schedules and enriching the newsletter with immensely informative articles.

Lastly, I would like to thank my entire editorial team for their persistent efforts in compiling this issue of CRS IC Newsletter. It has been a pleasure to bring this issue to life and we truly expect all our readers to enjoy reading it as much. Like always, constructive suggestions from our readers are welcome to aid us to surpass and elevate our efforts in future.

PROF. VANDANA B. PATRAVALE

CHIEF EDITOR, CRS IC NEWSLETTER

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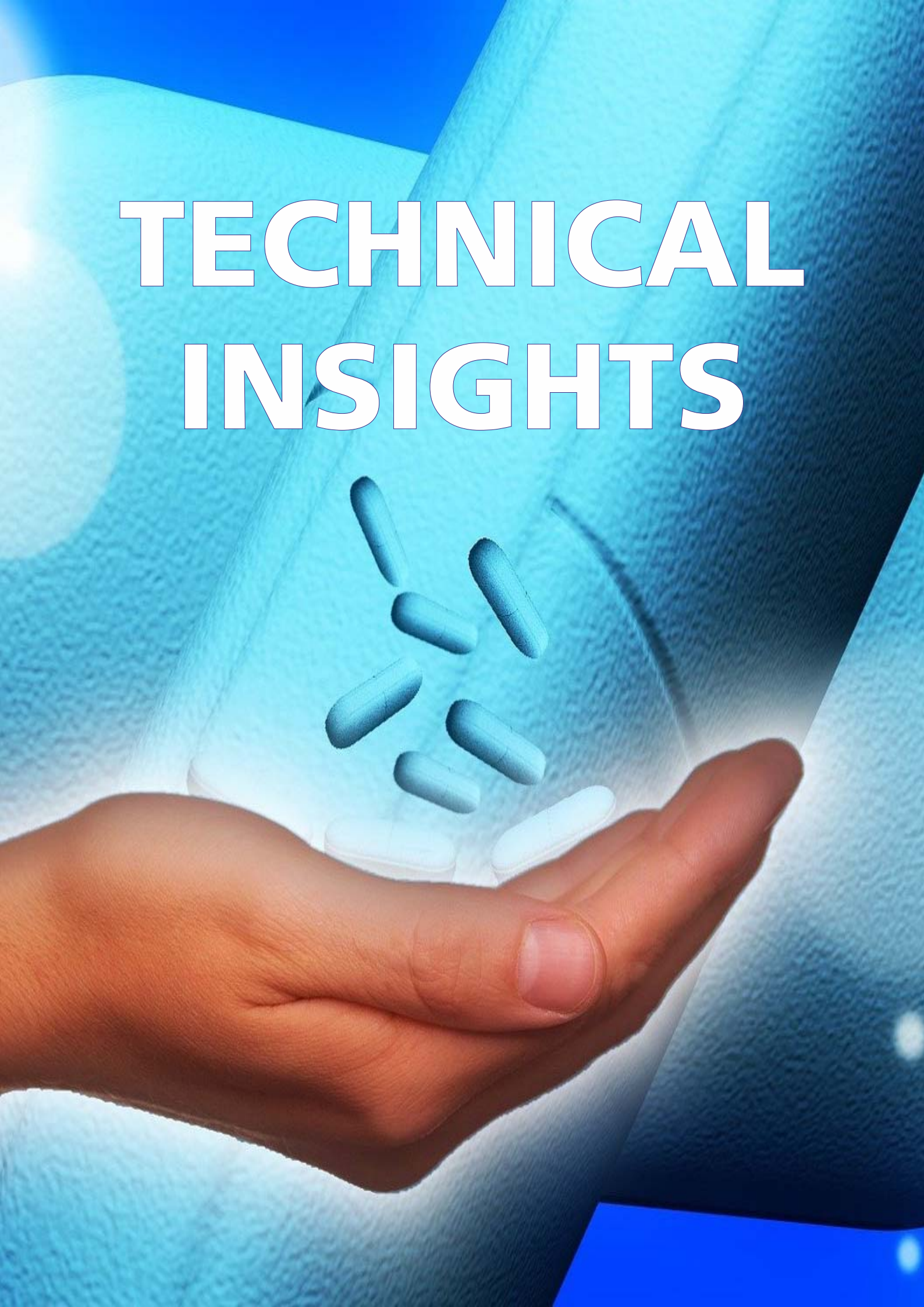
# TECHNICAL INSIGHTS



# Acetalated Dextran for Enhanced Delivery of Subunit Vaccines

**Erik S. Pena[a], Eric M. Bachelder[b], and Kristy M. Ainslie[a,b]***

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Acetalated dextran (Ac-DEX) is formed through the facile substitution of the pendant hydroxyl groups on dextran with acetal groups (1, 2). When generating Ac-DEX, the hydroxyl groups become modified with either the kinetically favored acyclic acetal or the thermodynamically favored cyclic acetal and the ratio of this coverage is controlled with reaction time (**Figure 1**). As the reaction is carried out for longer periods of time, higher fractions of cyclic acetals form, leading to a more slowly degrading polymer via hydrolysis (2, 3). Additionally, the pendant acetals afford acid sensitivity, wherein the polymer degrades more rapidly at lower pHs. Both the unique properties of tunability and acid-sensitivity have illustrated that Ac-DEX is especially advantageous in vaccine applications.

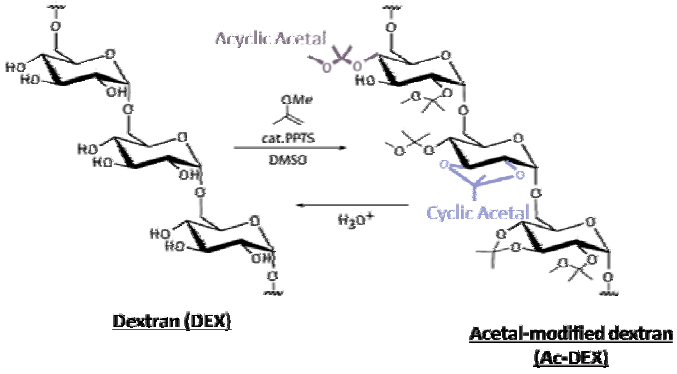
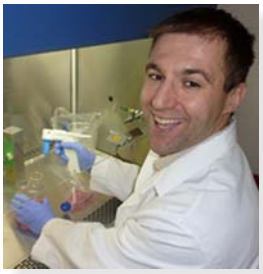



**Figure 1:** Ac-DEX Structure indicating acyclic and cyclic acetals

A common polymer used for degradable nano/microparticle (MP) based vaccine formulations is poly(lactic-co-glycolic acid) (PLGA), but Ac-DEX has several advantages over this polymer as well as other polyesters. For immune applications, an ideal polymeric MP would demonstrate stability at normal physiological pH, but once internalized by an antigen presenting cell (APC) it would break down and release the encapsulated cargo. Unfortunately, PLGA does not have that ability, whereas Ac-DEX is stable at normal physiological pH and degrades more rapidly when internalized by an APC due to its acidic phagosome/lysosome environment (1). Also, Ac-DEX's degradation products are pH neutral unlike polyesters (e.g., PLGA). Ac-DEX hydrolysis results in the release of ethanol, acetone, and soluble dextran. The amount of ethanol produced is exceedingly low and, in fact, the US Environmental Protection Agency reports pregnant women should have less than 50-300 mg ethanol/dL of blood, which would require greater than 35 grams of Ac-DEX (3), an impractical human dose. It has been calculated that only 1.1 grams is injected with US Food and Drug Association (FDA) approved polymeric formulation Vivitrol® (naltrexone), which would compare to the amount of Ac-DEX particles needed for a vaccine if scaled up to humans. This dose would result in a maximum release of acetone that is much lower than the 10-12 grams humans produce daily (4). For application in resource limited settings, having the ability to store vaccines outside the cold chain is strongly preferred. Ac-DEX MPs have been shown to maintain an encapsulate's activity at elevated temperatures (45°C) out to 90 days, whereas PLGA melts ~42°C (5).

Kristy M. Ainslie



Eric M. Bachelder

Erik S. Pena

In a subunit vaccine, there are typically two main agents: the protein antigen and the adjuvant. The protein antigen is a component of the pathogen with which the immune response is targeted, and the adjuvant activates the immune response to better prime the immune system towards the antigen. Ac-DEX has been shown to enhance the activity of both agents. Due to Ac-DEX's acid-sensitivity, protein antigen presentation is enhanced from APCs to T cells when delivered with MPs comprised of the polymer. Major histocompatibility complex I (MHC I) and MHC II presentation of protein antigens in Ac-DEX MPs have been shown to be significantly more efficient compared to polyacrylamide (another acid sensitive material), PLGA, and iron nanoparticles (3). This enhanced efficacy of the immune synapse is not a result of innate signaling from the polymer since previous experiments have shown that Ac-DEX alone does not stimulate a significant innate immunity both in vitro and in vivo (6-9). Also, dose sparing of an adjuvant can be achieved with acid labile Ac-DEX MPs with increased activity of the adjuvants imiquimod (10), R848 (11), murabutide (12), cGAMP (13), and CpG (14) observed in vitro and in vivo compared to soluble agents. When comparing the delivery of the adjuvant cGAMP, the Ac-DEX formulation drastically enhances innate activity greater than PLGA and a liposomal formulation (**Figure 2**) (13). Furthermore, it has been previously shown that Ac-DEX MPs encapsulating cGAMP retain their structural morphology and bioactivity following gamma irradiation with a sterilizing 25 kGy dose, allowing for post-manufacturing sterilization which can reduce overall production costs by not relying on a heavily sterilized clean room (**Figure 3**) (15).

In the use of Ac-DEX MPs for infectious disease vaccine applications, we have encapsulated protein antigens and the adjuvant R848 using an emulsion solvent evaporation process (16-18). Although vaccination with the emulsion MPs illustrated protection in a murine model against a lethal challenge from both Bacillus anthracis (causative agent of Anthrax) and separately Burkholderia pseudomallei (17, 18), high concentrations of neutralizing antibodies were not observed. Since denaturation of the protein antigen can lead to reduced concentrations of neutralizing antibodies, it was hypothesized that reducing

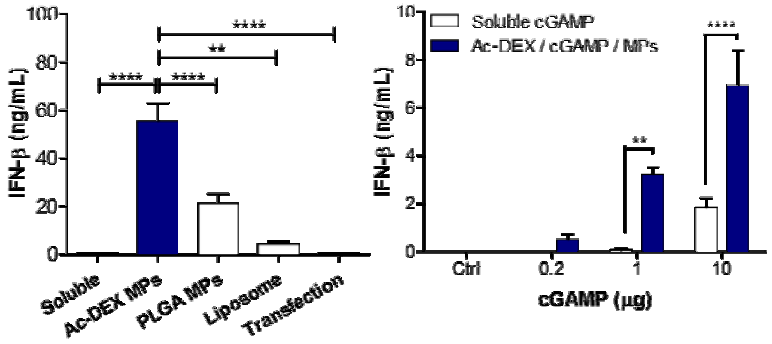



**Figure 2:** (left) BMDCs were treated with various cGAMP delivery vehicles, all at 1 μg/mL cGAMP, to assess IFN-β production or (right) C57BL/6 mice were injected intramuscularly and IFN-β production at the injection site was measured (26)

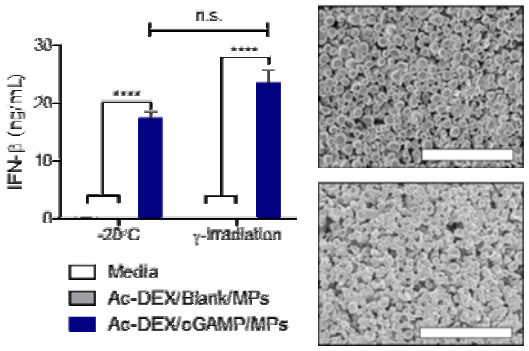



Figure 3. *In vitro* IFN-β production from BMDC's cultured with Ace-DEX/cGAMP/MPs untreated (-20ºC) or treated with 25 kGy sterilizing dose of -irradiation. SEMs of cGAMP MPs (top) before and (bottom) following -irradiation (scale bars=10 m) (15).

denaturation during manufacturing of the MPs would lead to an increase in neutralizing titers. Emulsion is known to denature proteins due to the extended contact time with solvents and high energy mixing. To evaluate this, the Anthrax vaccine antigen recombinant protective antigen (rPA) was encapsulated into emulsion and electrospray MPs. Electrospray, or electrohydrodynamic spraying, allow for the protein solution to be maintained in an aqueous phase and the polymer in an organic phase until they are expelled via low-flow syringe pumps through an electrically charged concentric capillary (19). The buffer/solvent can then evaporate in the aerosol phase, at which time the MPs solidify and are subsequently amassed on the grounded or oppositely charged collection plate. In the context of solvents, with Ac-DEX, the electrospray process used more benign FDA Class 3 solvents (e.g., ethanol, ethyl acetate) than Class 2 solvents (e.g., dichloromethane) that is commonly used in emulsions (20). Another ancillary benefit electrospray has over emulsion is that the spray heads can be multiplexed to create a continuous, non-batch, formation of MPs (21). When mice were vaccinated subcutaneously on day 0 and 21 with Ac-DEX MPs encapsulating rPA and/or R848 and lethally challenged with B. anthracis on day 28, a stark difference between protection from the emulsion and electrosprayed MPs was observed. There was significantly greater antibody titer as well as toxin neutralization with the electrospray MPs over emulsion MPs (16). Generation of MPs by electrospray has also led to higher encapsulation efficiencies of both rPA and R848, which allowed for the co-encapsulation of rPA and R848 into one MP. Interestingly, when comparing rPA and R848 delivered in a single MP (co-encapsulated) over co-injected in separate MPs, the separate MP group significantly outperformed the co-encapsulated group (**Figure 4**). It was then hypothesized that the unique signaling required for adjuvant activation and antigen trafficking was enhanced with separate particles and that this effect could be explored by evaluating the degradation tunability of Ac-DEX Mps.

To better understand the kinetics of antigen and adjuvant processing with MP formulations, the broad range of tunability observed with Ac-DEX's unique cyclic acetal coverage (CAC) can be exploited. In short, the higher the CAC, the slower the Ac-DEX degrades. For example, the degradation half-life at pH 5 for 20%, 40%, and 60% CAC is 0.25, 2.9, and 21.3 hours, respectively, keeping in mind that degradation is two logs slower at pH 7 (2, 22, 23). Generating Ac-DEX MPs of 20, 40, and 60 CAC that encapsulated the adjuvant murabutide and the model antigen OVA albumin separately, one could create MPs that would dictate the release of the protein antigen and adjuvant at different time intervals. Mice that were vaccinated on day 0 and 21 have shown that the serum total IgG responses for the encapsulated adjuvant murabutide peaked at medium CAC (40%) at day 42, whereas encapsulated antigen (OVA) has an inverse linear relationship with CAC, in that quicker degrading Ac-DEX resulted in higher titer (**Figure 5**). To evaluate the cellular response, IFN- production after antigen restimulation was noted to be inversely proportional to CAC for both OVA and murabutide particles when determined by an ELISpot and ELISA of splenocyte supernatants. To evaluate this in an infectious model, a universal flu peptide antigen (M2e) and adjuvant cGAMP

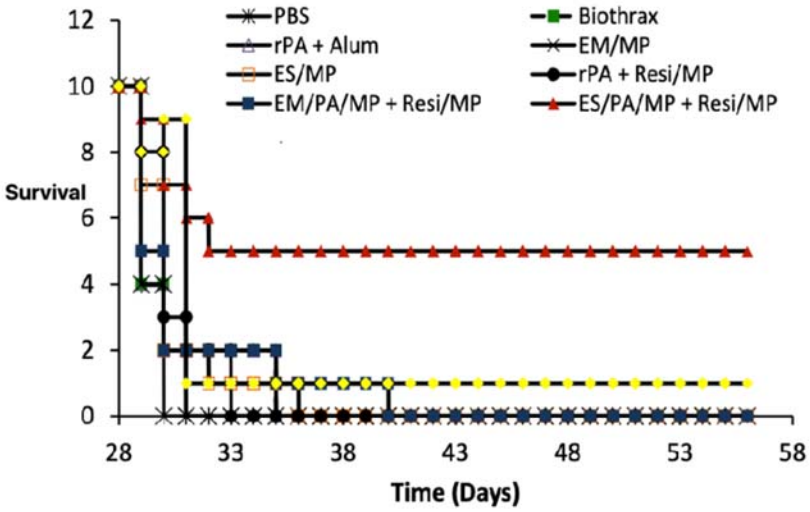



Figure 4. Vaccination of BALB/c mice with Ac-DEX MPs formed through emulsion (EM) and electrospray (ES) and then given a lethal IN challenge of *B. anthracis* (Ames strain, 100xLD50) (16). rPA = recombinant protective antigen. Resi = resiquimod (R848). Encapsulation in is indicated with /MP.

was encapsulated in MPs of different CACs of Ac-DEX. As with OVA and murabutide, the CAC of the antigen or adjuvant particles significantly affected total IgG titers, antibody isotypes, IFN- production, and protection against lethal challenge. However, the optimum CAC for these antigens and adjuvants combinations were unique from what was observed with OVA and murabutide. This indicates that each antigen and adjuvant have unique immune activation rates that can vary within the degradation time-frame available with Ac-DEX. Of note,

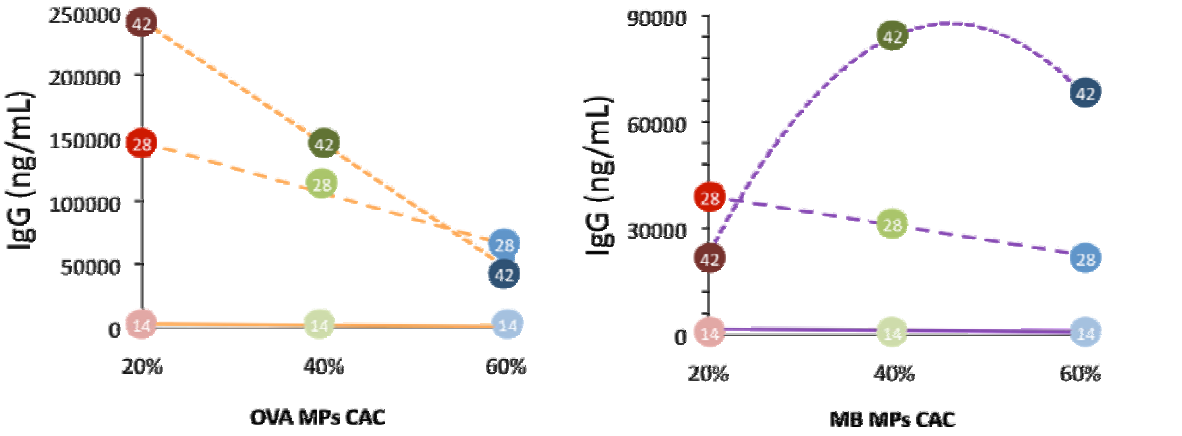



Figure 5: Total IgG from C57Bl/6 mouse sera collected on Day 14, 28, and 42 (indicated in circles) post vaccination (day 0, 21) with either (left) OVA MPs co-delivered with soluble monophosphoryl lipid A or (right) encapsulated murabutide (MB) delivered with soluble OVA (12).

the separate Ac-DEX MPs protected more effectively and produced more broadly active antibodies than co-encapsulated M2e and cGAMP (13), which may or may not be supported by work with other platforms(25).

Overall, Ac-DEX has unique properties imparted by its acid-sensitivity and degradation tunability. These properties have indicated enhanced antigen and adjuvant delivery when Ac-DEX MPs are used as a vaccine formulation. Additionally, the use of minimal-denaturing fabrication methods, like electrospray, to make Ac-DEX MPs that encapsulate protein antigen led to an increase in protection and neutralizing titer. Lastly, the fabrication of antigen and adjuvant in separate particles has illustrated enhanced protection compared to co-encapsulated particles. In conclusion, Ac-DEX MPs have demonstrated great potential to be a significant platform for the generation of infectious disease vaccines for the future.

## Abbreviations:

| | |
|---|---|
| Ac-DEX | : Acetalated dextran |
| APC | : Antigen presenting cell |
| CAC | : Cyclic acetal coverage |
| FDAUS | : Food and Drug Administration |
| MHC | : Major histocompatibility complex |
| Mps | : MicroparticlesOVAOvalbumin (egg white) |
| PLGA | : Poly(lactic-co-glycolic acid) |
| rPA | : Recombinant protective antigen |

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## QUIZ-I

**1. How much glucose is needed daily for brain?**

a) 120g b) 100g c) 125g d) 110g

**2. ANVISA is the pharmaceuticals regulatory body of**

a) USA b) Japan c) Brazil d) Portugal

**3. The first case of SARS CoV-2 was identified in which country?**

a) Somalia b) China c) USA d) Russia

**4. USP Type-IV dissolution apparatus is commonly known as:**

a) Flow-Through Cell b)Paddle Type Apparatus
c) Reciprocating Cylinder d) Paddle over Disk

**5. Which Indian company is manufacturing the "Covishield" vaccine?**

a) Bharat Biotech b),Serum Institute of India
c) Zydus-Cadila d) Syngene

**6. Avicel PH-102 contains which of the following?**

a) Silicon dioxide b) Dicalcium Phosphate
c) Microcrystalline Cellulose d) Starch

**7. The calibration of dissolution testing apparatus can be done using what?**

a) Ibuprofen tablets b) Prednisolone tablets
c) Pantoprazole tablets d) Paracetamol tablets

**8. Jennifer Doudna & Emmanuelle Charpentier won the 2020 Nobel Prize in Chemistry for which of the following discoveries?**

a) CAR-T Cell Therapy b) CRISPR-Cas9
c) Lithium-ion battery d) Discovery of Hepatitis C virus

**9. Which of the following diagnostic test is used for leprosy?**

a) Schick test b) Mantoux test
c) Lepromin test d) Widal test

**10. The word '*quarantine*' comes from**

a) The number 40 b) The term guarantee
c) The fraction quarter d) A quarter of the year

# "Tumor-on-chip" for Personalized Medicine

**Eliana Steinberg and Ofra Benny***

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Eliana Steinberg

Ofra Benny

Despite the great volume of research and investment devoted to the development of new therapies, cancer remains having a high mortality rate of approximately 10 million deaths in 2020 worldwide (1). Pre-clinical models have a limited ability to accurately recapitulate tumors and their microenvironment (TME), consequently leading to the discrepancy between drug effectiveness in pre-clinical models versus clinical trials (2). Thus, there is a pressing unmet clinical need to develop experimental models that accurately mimic tumors. Tumor tissue is comprised of several components that generally can be categorized into two: cellular and acellular. The various cells that make up a tumor and its surrounding environment (i.e., cancer cells, cancer-associated fibroblasts, endothelial cells, immune cells, etc.) as well as its acellular components – such as the extracellular matrix (ECM), basement membranes and cytokines – strongly influence tumor progression and its pharmacological response to therapy (Figure. 1) (3-5). Currently, the most prevalent means used for testing tumor responsiveness to drugs prior to in vivo studies is the two-dimensional (2D) cell culture. However, monolayer culture of cells generally fails to reliably reflect the patient's complex TME because it cannot display three-dimensional (3D) key features, such as spatial cell-cell or cell-extracellular matrix (ECM) interactions (2,6-8). Additionally, 2D culturing systems are insufficient in reproducing the intratumoral heterogeneity and rapid adaptation of the tumor to its surroundings (9,10).

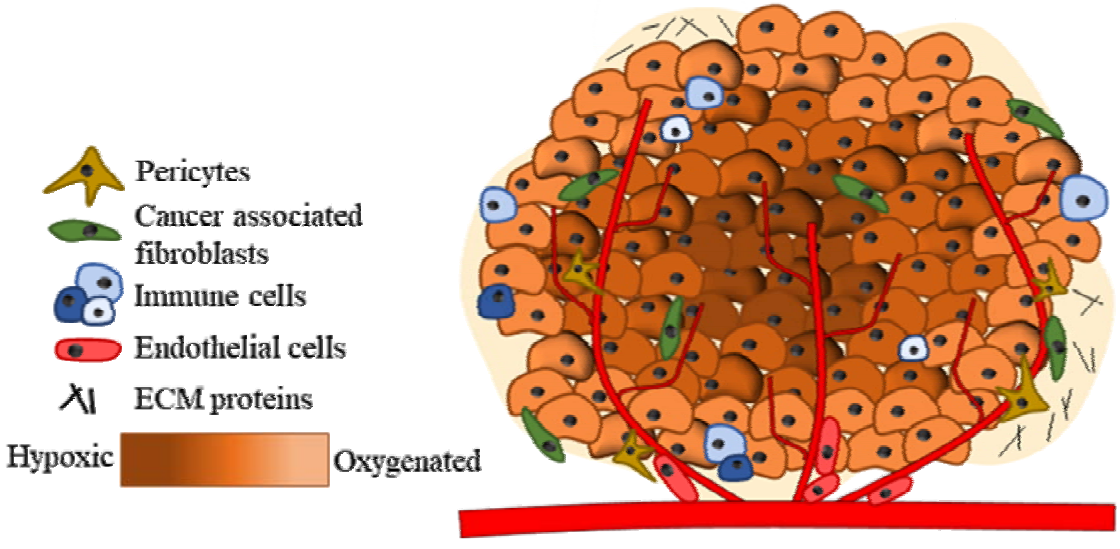


Figure 1. The tumor and its microenvironment. The tumor microenvironment is comprised of several cell and acellular components including cancer cells, cancer-associated fibroblasts, endothelial cells, immune cells, etc. as well as acellular components such as the extracellular matrix (ECM). Beyond a size of ~200 micron diffusion is limited and as a results hypoxia is developed in the core. Long term hypoxia may lead to necrosis.

The importance of 3D culturing for drug screening research is increasingly recognized (11). Tissue specimens, organoids and spheroids are used in cancer research as an intermediate model between cancer cell lines in vitro and tumor xenografts (12-14). Cancer cells that are grown in 3D retain important physiological aspects such as a dynamic metabolic demand, hypoxia and TME

interactions that more closely resemble those of the parent tumor compared with cells grown in 2D (15,16).

An advanced approach in ex vivo 3D culturing is obtained using "organ on chip" technology – micro-engineered devices that biomimic the function, biochemistry and mechanical cell strain of various living organs. Adding flow to these devices elicits mechanical forces, such as fluid shear stress and hydrostatic pressure, which have a significant impact on cancer behavior (17). In cancer research, microfluidic chips enable the recapitulation of tissue–tissue interfaces and the physiological relevant microenvironment organization while sustaining perfusion in vitro. Microfluidic cell culture devices are comprised of optically clear materials (e.g., plastic, glass, silicone, flexible polymers, etc.) that contain microchannels populated with cells. These devices are fabricated using different methods, such as injection molding, photopolymerization, 3D printing and other microscale manufacturing techniques (18). The accurate control of cell culture conditions can ideally allow the continuous transport of nutrients and oxygen, as well as the removal of cellular waste products, enabling a better control over local biochemical gradients similar to the TME (19-21). Fine control over flow rates can subject the culture to different flow patterns, thus incorporating mechanical forces that can substantially mediate tumor progression (22). The technological advantages include low-volume usage, screening in a high-throughput manner, and obtaining rapid results (23).

Due to the many complexities and substantial heterogeneity of cancer (24,25), the development of reliable tumor tissue culture models that can mimic malignancy behaviors more accurately is of great value. Additionally, the incorporation of an appropriate microenvironment that can mimic immunological responses is greatly needed, since this is known to strongly affect tumor behavior and progression (26). Personalized microfluidic models can be clinically relevant as predictive drug-performance tools, enabling doctors to prescribe the most effective treatment for each patient without having to engage in trial and error (Figure. 2).

The emergence of "tumor-on-chip" technologies opened the possibility of maintaining patient specimens long-term in a physiologically mimicking manner in order to support basic cancer studies as well as high-throughput drug screening. The high versatility of the microfluidic platform may provide unique features for each individual


Figure 2. "Tumor-on-chip" technology for precision medicine. Maintaining 3D culture samples derived from patient specimens in a device which supports a long-term growth in a physiologically mimicking manner provides a useful platform for high-throughput drug screening for personalized medicine.

case. For example, xenograft-originated sections were shown to survive more than eight days under low-shear stress conditions and preserve cell composition similar to the original patient-derived ovarian and prostate tissues (27). Interestingly, the intra-tissue heterogeneity led to distinct chemotherapy responses within a single specimen.

A further advance in mimicking the physiological TME is the incorporation of immune system components, which are highly relevant for treatment efficacy, especially given the recent success of immunomodulating drugs in oncology (28). Tissue models in the form of tumor spheroids retaining various immune cell populations in a short-term culture proved to be pathophysiologically relevant and maintained tumor cell dynamics and heterogeneity (29-31). However, it should be noted that such models are limited to preexisting tumor-infiltrating immune cells and do not include the recruitment of additional immune cells into the model, thus neglecting indirect effects of the preexisting immune cells. This system, with a few adjustments, has the potential to provide a powerful means for personalized immunotherapy.

Cancer metastasis to distant organs is the leading cause of cancer-related death (32) and occurs when circulating tumor cells extravasate and colonialize in distant tissues. Thus, assessing the metastatic potential of a tumor on a chip can be highly efficacious. Several studies attempted to mimic various aspects of metastases (33-36). For example, it was shown that cells can be sorted based on their capacity to travel through small constrictions, as occurs in metastases (37). Such systems may be adjusted for high-throughput separation of cancer cells based on their size and mechanical-phenotype. Based on this, microfluidic platforms may be used for studying the underlying molecular mechanisms in metastasis and for predicting therapeutic effects at different disease stages (38,39).

A critical aspect in drug efficacy is the examined sample's cell composition, since the TME's cells strongly affect tumor behavior and response to therapy (35). For example, it was shown that co-culturing primary lung cancer cells with primary pericytes on a chip at various ratios influenced the effect of the drugs (40). It was proposed that pericytes can act as a protective barrier around the primary lung epithelial tumor spheroids against chemotherapy effects. Similarly, tumor spheroids co-cultured with cancer-associated fibroblasts showed a higher survival advantage compared with monocultured tumor spheroids exposed to chemotherapy treatment (41,42). This indicates that "tissue-level" resistance can be identified, in addition to the innate tumor cell resistance to drugs, which substantially expands the definition for drug screening.

Our recent study showed an example of how an ex vivo model may detect clinically relevant information (43). In this study, exome sequencing was performed on patient-derived glioblastoma spheroids grown for several days, leading to the discovery of subclonal driver mutations that were undetected in the parent tumor when using standard depth of sequencing. They identified an important targetable resistance mutation in lung cancer, EGFR T790M, that was not previously described in primary glioblastoma. The ability to analyze tissues grown ex vivo enabled a more extensive genetic analysis that provided useful clinical information.

## Conclusion:

Microfluidics potentially offers many benefits. It can:

- Open up new avenues in the basic research of tumor biology;
- Be used for drug screening in patient-derived tumor cells and "point of care" diagnostics for personalized therapy;
- Be used to determine the most effective treatment prior to administration to the cancer patient;
- Predict a drug's success in eradicating a patient's tumor in a cost-effective manner;
- Increase treatment efficiency;
- Reduce a patient's suffering and unnecessary adverse side effects.

Standardizing the tumor-on-chip systems and allowing for high-throughput screening while still maintaining tumor complexity and heterogeneity remain challenges for the future. It may be essential to merge the different 3D microfluidic systems into one to overcome these challenges and produce a clinically relevant tumor model. Utilizing advanced manufactory technology, such as 3D printing, for better tuning and accessibility of these important tools may further advance cancer research.

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# Stimulator of Interferon Genes (STING) - Agonist Delivery Strategies and Clinical Potential

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It is increasingly evident that the tumor microenvironment is strongly immunosuppressive and is a critical determinant in the efficacy of immunotherapy. Therefore, mitigating tumor immunosuppression using the innate immune system is an attractive approach in cancer immunotherapy. Engagement of pathogen-associated molecular patterns (PAMP) with their respective pattern recognition receptors (PRRs) can elicit robust downstream endogenous cytokine production and immune cell activation (1), which is responsible for the potent immune responses generated by vaccines and against tumors. Cyclic dinucleotides (CDNs), such as the second messenger 2'3'-cyclic guanosine monophosphate-adenosine monophosphate (cGMP-AMP or cGAMP), are a class of PAMP that are generated upon sensing cytosolic DNA (2,3). The production of cGAMP leads to agonism of stimulator of interferon genes (STING) (4,5), enacting a type I interferon (IFN-I) driven proinflammatory program including the stimulation of dendritic cells (DCs) and cross presentation of tumor antigens to T-cells, thereby priming them for antitumor effector functionality (6,7). Several STING agonists have been developed and have demonstrated promising results in different pre-clinical models of cancer, with lead candidates progressing to clinical trials. In addition, co-administration of STING agonists with other immunotherapeutic entities, such as cancer vaccines, immune checkpoint inhibitors, and adoptive T cell therapies are encouraging combinatorial approaches for cancer treatment.

Despite promising pre-clinical findings, delivery of STING agonists, particularly CDNs, present with numerous challenges. Endogenous CDNs contain a phosphodiester bond that is degraded by phosphodiesterases such as ectonucleotide pyrophosphatase / phosphodiesterase (ENPP)1, rendering them inactive. In addition, CDNs are anionic and highly hydrophilic which restricts their entry into the cytoplasm where STING resides (8). Intratumoral (IT) injection of exogeneous STING agonists is a logical approach to achieve high tumoral concentrations, however these molecules are rapidly eliminated from the tumor and thus only transiently interact with the immune cell component of the TME. Therefore, high CDN doses are needed to achieve adequate biological activity, which can result in a negative impact on antitumor immunity due to the induction of programmed death-ligand 1 (PD-L1) on tumor cells, increased tumor-in ltrating regulatory T cells (Tregs) and granulocytic myeloid-derived suppressor cells (MDSCs). Although small molecule STING agonists have been developed for oral and intravenous administration, their potency compared to CDNs is under examination, and there are concerns surrounding dose limiting toxicities and rapid metabolism that occur with these delivery routes.

## How biomaterials can be used to improve STING agonist therapeutic efficacy

Biomaterial-based delivery strategies can be leveraged to overcome some delivery challenges. Different approaches can be used to further improve STING agonist activity; however, the tumor biology, stage and localization will dictate the final biomaterial design.

Nanoparticles (NP) have emerged as suitable vehicles for drug delivery as they enable overcoming biological limitations and mitigating drug toxicities. NP-CDN formulations enable the protection of STING agonist and improve its internalization into specific cells, therefore augmenting its activity (9,10). Nowadays, systemic delivery of nanoparticles is the main delivery route for non-accessible or metastatic tumors. In contrast, to treat accessible tumors, injectable hydrogels or microneedles-based platforms embedded with CDN nanoparticles are also promising biomaterials platforms.

Previous efforts for the intratumoral (IT) delivery of CDNs centralized around encapsulation-based

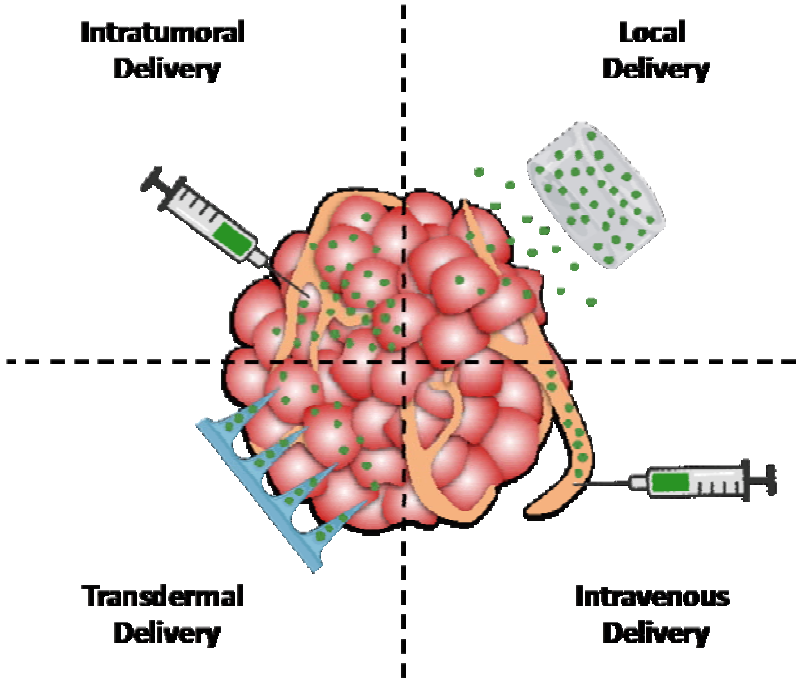


Figure 1: Potential delivery routes for STING agonist using biomaterials.

strategies whereby the CDN is housed inside of the NP. For instance, liposomes (11-13) and polymeric NPs such as polymersomes (14), PLGA (15) and acetylated dextran (16) have been used for the IT administration of CDNs. These formulations typically rely on intrinsic elements of the synthesized material (for example, pH, redox potential and enzymes) for effective delivery. Although the IT delivery approach is attractive, this approach is generally limited to accessible tumors, and repeated CDN administrations is invasive. Local delivery as alternative delivery route, can afford more sustained release of STING agonist, avoiding the need of multiple administrations. In addition, local delivery platforms can be leveraged to combine multiple therapies. An innovative approach was recently outlined whereby a hydrogel was created using CpG DNA and loaded with the stimulator of interferon genes (STING) agonist cyclic-di-GMP and finally coated with melanin. This system, comprised of two separate adjuvants and melanin, could provide heat-induced immunogenic cell dead (ICD) upon near-infrared (NIR) irradiation. Local administration of this hydrogel, along with NIR irradiation, was found to eliminate primary tumors with evidence of DC maturation in the tumor draining lymph node (tdLN) and accompanying TME changes such as increased cytotoxic T-cells and decreased Tregs (17).

Transdermal drug delivery has captured researchers' attention in the past few years. Particularly, microneedle-based devices can be used to efficiently deliver a large range of therapeutic agents, such as CDN, along with combination therapies. MNs possess many outstanding properties, such as the ability for painless traverse of the stratum corneum, minimal invasiveness, excellent biocompatibility, and minimal side effects. MNs have been extensively used for the delivery of immunomodulatory drugs. For instance, the delivery of nano-micelles encapsulated with R848 within dissolvable MNs has been shown to promote nanoparticle migration to the lymph node and induce antigen-specific humoral and cellular immunity. Increased levels of INF-α+CD8+ T cells and reduction in tumor size were found in mice (18). Even though these approaches provide with great opportunity for local treatment of accessible tumors, their success is predicated upon the ability to generate a systemic anti-tumor immune response. Several studies have shown that local immunostimulants delivery only induce local immune responses at the injected site, with limited effect on distant tumors, which limits its applicability in patients with advanced stage tumors, which present metastatic lesions (19,20). Systemic delivery of immunostimulants can overcome some of these limitations, where CDN can be delivered into primary and metastatic lesions. However, the delivery of unprotected STING agonists may lead to rapid absorption into the blood causing a systemic cytokine storm that can be harmful (21) . Nanoparticle-based targeted delivery can reduce off- target toxicity and immune- related adverse events. For instance,

liposomes (22) and polymersomes (14) have been used for the systemic delivery of CDNs. These nanoparticles allow the encapsulation and protection of CDN molecules from the enzymatic degradation upon administration. In addition, CDN-NP formulations improve the pharmacokinetics of CDN drugs allowing higher tumor accumulation. After CDN-NP administration, STING was found to be activated in the tumor which was theorized to be responsible for the altered TME including increased numbers of $CD4^+$ and $CD8^+$ T-cells as well as DCs accompanied by decreased numbers of macrophages. Pronounced therapeutic efficacy and increased survival was observed in different tumor models (22).

IT injections of free cGAMP. The TME was reprogrammed in treated mice, as was demonstrated by increased $CD8^+$, $CD4^+$ T cells, including memory linages as well as NK cells and activated DCs. Furthermore, the macrophage population in the TME was repolarized to a more pro-inflammatory phenotype. The applicability of the system was further extended as other therapeutic moieties such as CpG or the chemotherapy agent, pemetrexed, were also shown to exhibit a pulsatile release profile (26). Exploring this concept further could involve directly comparing pulsatile and sustained release profiles of STING agonists to determine the optimal regimen based on readouts such as therapeutic efficacy and memory response, as the dose of CDN has been shown to dictate

**Table 1: Biomaterials for STING agonist delivery**

| Administration Mode | Material | STING payload | Dose | How biomaterials help? | Reference |
|---|---|---|---|---|---|
| IV | Liposomal NP | 3'3'-cGAMP | 10 μg | Blood stability | 22 |
| IV | Polymersomes NP | 2'3'-cGAMP | 20 μg | Blood stability; endosomal scape | 14 |
| IT | pBAE NP | ADU-S100 | 2 μg | Increase cell internalization and endosomal scape | 23 |
| IT | Cationic silica NP | c-di-GMP | 5 μg | Local inflammation and efficient drug release | 24 |
| IT/ Local | Peptide Hydrogel | ML RR-S2 CDA | 20 μg | Prolong STING agonist in the TME | 25 |
| IT | PLGA Microparticles | 3'3'-cGAMP | 40 μg | Controlled pulsatile release | 26 |

## How biomaterials can be used to explore STING-induced immunity

It is evident that biomaterials can act to augment the intracellular concentrations of STING agonists such as CDNs either within the TME or secondary lymphoid organs such as the spleen, resulting in considerably improved therapeutic efficacy across multiple pre-clinical tumor models. However, biomaterial-based systems can also be used to answer immunological questions that are difficult answer otherwise. For example, the effect of release profile of CDN on therapeutic outcomes can be studied using materials; biodegradable PLGA microparticles (MPs) were designed to enable pulsatile release of cGAMP following a single IT injection using three different variants of PLGA MPs to permit differential release of cGAMP, a few days apart, due to the composition of each type of MP, The differential MP degradation rate dictated cargo release profile. Indeed, a single injection of MPs prolonged survival of melanoma and breast tumor-bearing mice to the same extent as four

the type of STING-mediated immune response, either memory-forming or ablative without efficient memory generation (27). As previously alluded to, functionalization of NPs with targeting moieties may be able to guide cargo to different cell types, which would allow deconvolution of the role of specific cell types in STING-based immunity, and their interactions, aided by techniques such as intravital microscopy. Indeed, the kinetics and cellular involvement of a curative versus non curative immune response are likely to differ substantially, and deeper understanding these differences could lead to more refined and effective STING-based therapies. An extension of this could involve the study of combination of STING agonism and another therapeutic modality such as chemotherapy or photothermal therapy, which are used clinically and can be delivered or induced respectively, using biomaterials.

## Perspectives and conclusions

The popularity of STING as a therapeutic target in cancer

immunotherapy is a testament to the potent proinflammatory cascade that is elicited upon its activation. Drawing on lessons from viral immunology, it was discovered that STING activation could induce antitumor immunity based on the downstream events that follow such as type I interferon production, innate immune cell activation and cross presentation of antigen to T-cells. Consequently, synthetic analogs of STING agonists such as CDNs have been generated that possess striking potency. Due to difficulties achieving therapeutic intracellular concentrations of CDNs, biomaterial-based strategies have been implemented to great effect in preclinical murine models of cancer, particularly in combination with immune checkpoint blockade, typically requiring less than 10 micrograms to achieve therapeutic efficacy. As STING agonists are currently being tested in clinical trials, there will still be a significant period of time until biomaterial-based STING therapies reach the clinic. This provides researchers with the opportunity to continue to explore how biomaterials can be used to improve the therapeutic efficacy of STING agonists *via* different delivery routes, and aspire to utilize and seek synergistic combinatorial approaches, informed by tumor biology (e.g. cold or hot tumor). If the side effects of STING agonism, when given systemically, can be attenuated, this would further improve and expedite the translation process. Studies elucidating the precise mechanisms by which biomaterials potentiate STING agonism, or can provide insight into the underlying immunobiology which dictates efficacy and robust memory responses would be valuable to the field.

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# Changing Paradigm in Computational Pharmaceutics

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The application of computational modelling to drug delivery and pharmaceutical formulations, termed "computational pharmaceutics" is getting increasing attention because of its enormous potential for developing rational, deductive and knowledge based strategies in pharmaceutics. The formulation development process always remains challenging due to its complex, tedious, time-consuming nature, which involves several steps and requires an enormous amount of resources to develop a stable dosage form (1). Therefore, there is a great scope to improve mechanistic understanding to simplify the process of formulation development wherein computational interventions can play a pivotal role. Combining existing pharmaceutics branches, Computational Pharmaceutics which includes the use of computational algorithms, can be of great help to reveal mechanistic details of pharmaceutical processes and formulation development strategies.

With modern advances in computational algorithms, it is possible to simulate and model complex systems that earlier were difficult to measure and model experimentally. With the development of high-performance computing, multiscale modelling techniques have been widely pursued, from quantum mechanics (QM) and molecular mechanics (MM) simulations to stochastic Monte Carlo methods, coarse-grained molecular dynamics (CG-MD), discrete element methods (DEMs), finite element methods as well as advanced analytical modelling (2). Various modelling techniques used

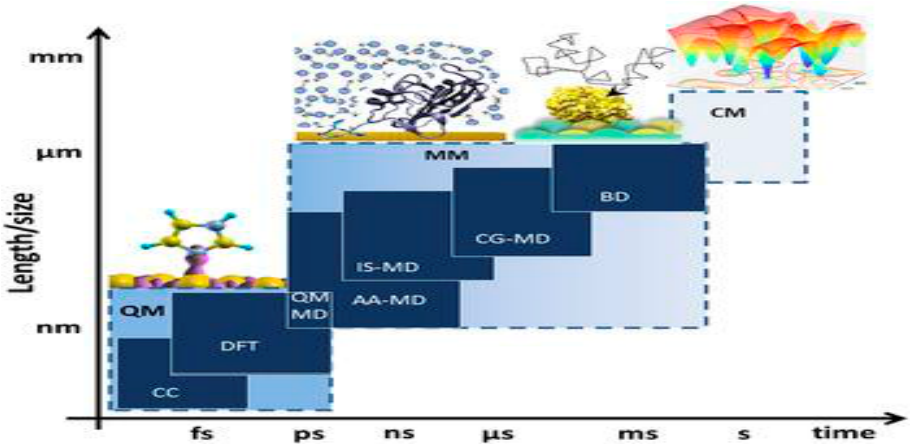


Figure 1: Applications of modelling techniques in computational pharmaceutics

*[Adapted with permission from Cambridge University Press (3)]*

in computational pharmaceutics are summarized in Figure 1.

Majorly, molecular simulations are used to mimic the physical motions of atoms and molecules using Newton's law of physics for large systems containing millions of atoms –(4). The basic idea of any simulation is to investigate a particle-based system after constructing it and generate a trajectory describing the evolution of the system over time (5).

Depending on how the system is propagated, simulations can be classified as Molecular dynamics (MD) and Monte Carlo (MC). Usually, atomistic MD simulations are used to generate a dynamical trajectory of the system and are used widely in simulation algorithms (6). The basic steps followed to

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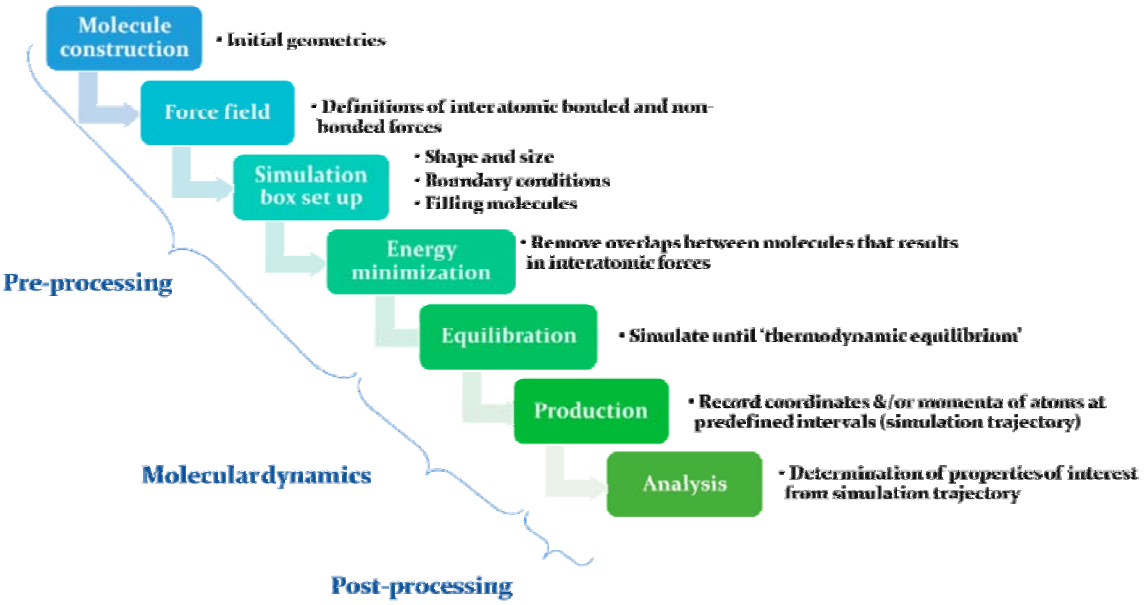


Figure 2: Schematic representation for atomistic molecular dynamics simulations.

perform atomistic MD simulations are summarized in Figure 2.

The current article aims to give a brief overview of computational methodologies concerning pharmaceutics.

**Preformulation:**

Preformulation is the development stage during which physicochemical properties of the API and excipients are screened to obtained data to determine the appropriate course of formulation development. Studies relating to drug solubility, solubilization, partitioning, compatibility between polymers, drug-excipient compatibility, co-crystal screening, etc., have been reported using *in silico* approaches.

The solubility of a drug in any physiological media is crucial for its bioavailability and therapeutic activity. Solubilization, the process leading to solvation of a solute in some solvent, is essentially a two-stage process. Solubilization requires bonds between solute molecules in their solid form to be broken, followed by the formation of cavities in the solvent in which solute molecules insert themselves and interact with the surrounding solvent (7). This behaviour of solute-solvent interaction can be observed using MD simulations.

One of the significant practical approaches for solubility enhancement is to complex the poorly soluble agent with cyclodextrins. Researchers have successfully worked on studying the interactions between the drug and cyclodextrin computationally. Kumar et al. studied the interactions of different NSAIDs drugs with -cyclodextrin, stating a good interaction between the drugs and cyclodextrin (8). Hossain et al. demonstrated the use of free energy calculations to determine solubility. They noted that the relative solubility knowledge when combined with calculated solvation free energies and experimental understanding of the solubility in one reference environment, will pave the way to calculating absolute solubilities in any new solvent (7). Thus, free energy calculations combined with MD simulations can be used to estimate and predict solubility. Martin et al. used the same free energy perturbations to calculate benzocaine and phenytoin's partitioning across the lipid bilayer. This factor is critical to their mode of action (9). Researchers have also explored the formation and solubility of co-crystals using OPLS-AA forcefield(10).

Thus, the preformulation screening can be done at ease without any laborious work and loss of material using the computational tools.

**Computational approaches to study drug delivery systems**

Computational methods that can be used to study critical aspects of drug delivery system prior to experiments, are increasingly desirable to minimize the investment in formulation development. In the recent past, significant progress in MD simulation methodologies has been made to study drug delivery. Such methods are directly applicable to the design and optimization of drug delivery systems. As shown in Figure 3, MD simulations are particularly valuable in addressing issues that are difficult to be explored in laboratory experiments for drug delivery. Nanoformulations such as nanoemulsion, polymeric micelles, liposomes and dendrimers are promising drug delivery systems, whose selection and

optimization can be gainfully conducted by theoretical methods. Molecular modelling has turned out to be the critical approach for understanding the structural and functional attributes of the dendrimer to rationalize its design (11). Suek and Lamm et.al used MD simulations to study dendrimers with mixed solvophobic and solvophilic caps using a coarse-grained (CG) model (12). Lee et al. have simulated polyamidoamine (PAMAM) dendrimers with 90% acetylated chain termini. To improve the prospects of utilizing dendrimers, it is essential to have a good understanding of their structure with respect to various modifications (13). The liposomal surface can be engineered and functionalized by selecting special lipids, in facilitating covalent binding of proteins (e.g. antibodies and proteins to sugars, *i.e.* lectin), glycoproteins and synthetic proteins (14). MD simulation is helpful to open new opportunities to investigate liposomes structure and functionality further. Coarse-grained (CG) models offer a simulation of larger systems like lipids for longer times by decreasing the number of degrees of freedom (df) compared with all-atom models (15). To simulate membranes and membrane-related phenomena, the MARTINI CG force field appears to be the best choice because the membranes are under the action of amphipathic assembly forces. The MARTINI force field is, therefore, the most suitable field for modelling such systems. Within the framework of this model, the molecules are not represented by individual atoms, but rather by "pseudo-atoms" approaching groups of atoms, such as whole amino acid residues. By decreasing the freedom degrees, much longer simulation times can be studied at the expense of the molecular details (16). Molecular simulations have been employed to investigate the structure, dynamics, and self-aggregation properties of polymeric micelles, with or without drugs (17). Huang et al. investigated glycyrrhetinic acid-modified poly(ethylene glycol)-b-poly( -benzyl L-glutamate) (PEG-b-PBLG) micelles as drug carriers for doxorubicin (18).

## Case studies:

In addition to different design aspects of drug delivery systems, computer simulation has been used to study how chemical modification and surface functionalization influence their interactions with the biological membrane. Herein we are discussing two interesting case studies wherein computer simulation has been used to get significant insights into how structural attributes of nanoparticles affects specific interactions between receptors and ligands.

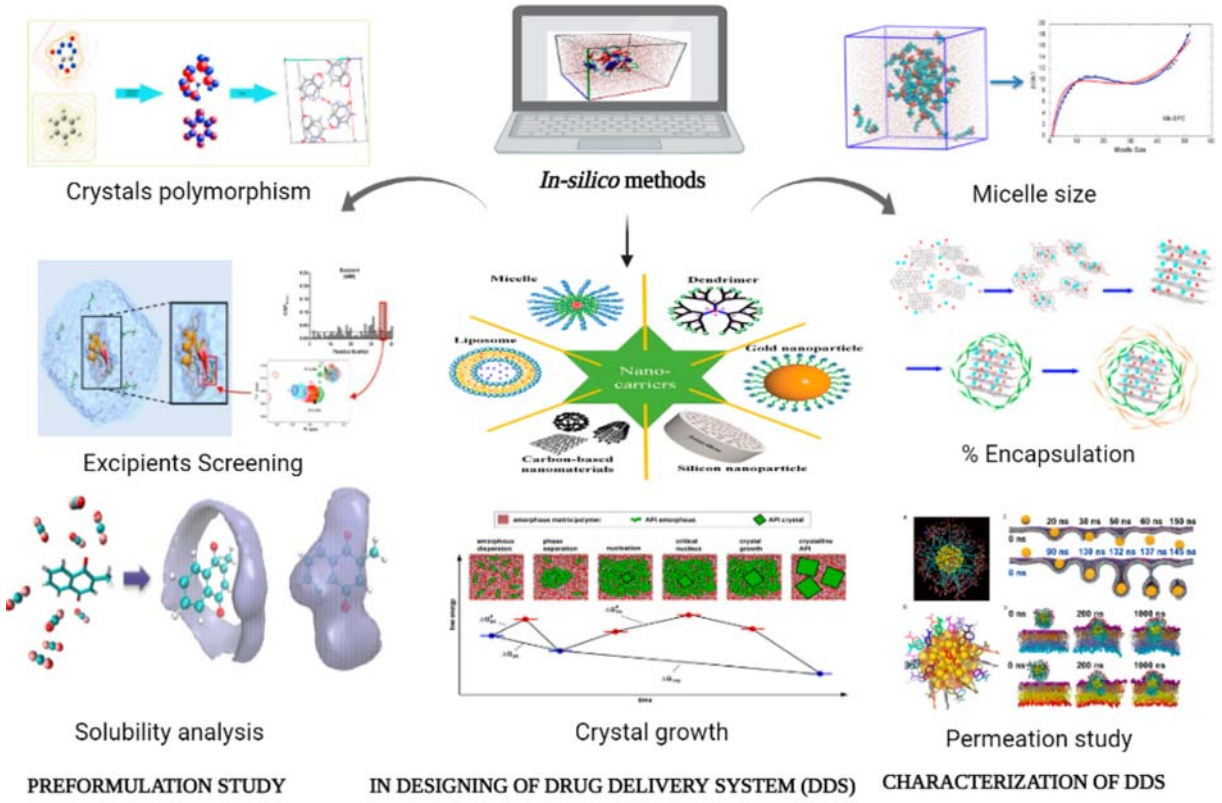


Figure 3: A schematic representation of applications of computational pharmaceutics

### *a) Understanding cellular uptake of nanoparticles with pH-sensitive polymers*

Ding et al. systematically studied the receptor-mediated endocytosis of nanoparticle-polymer complex (NPC, nanoparticle with pH-sensitive polymers absorbed on its surface) using DPD simulations (19). The endocytosis process of NPC depends on pH environments. As shown in Figure 4, the nanoparticle can only be engulfed by the cell membrane when the pH is higher or lower than the polymer's pKa, whereas endocytosis is blocked when the pH equals the polymer's pKa. This happens because externally adsorbed macromolecules on the nanoparticle surface suppress the specific interactions between receptors and ligands.

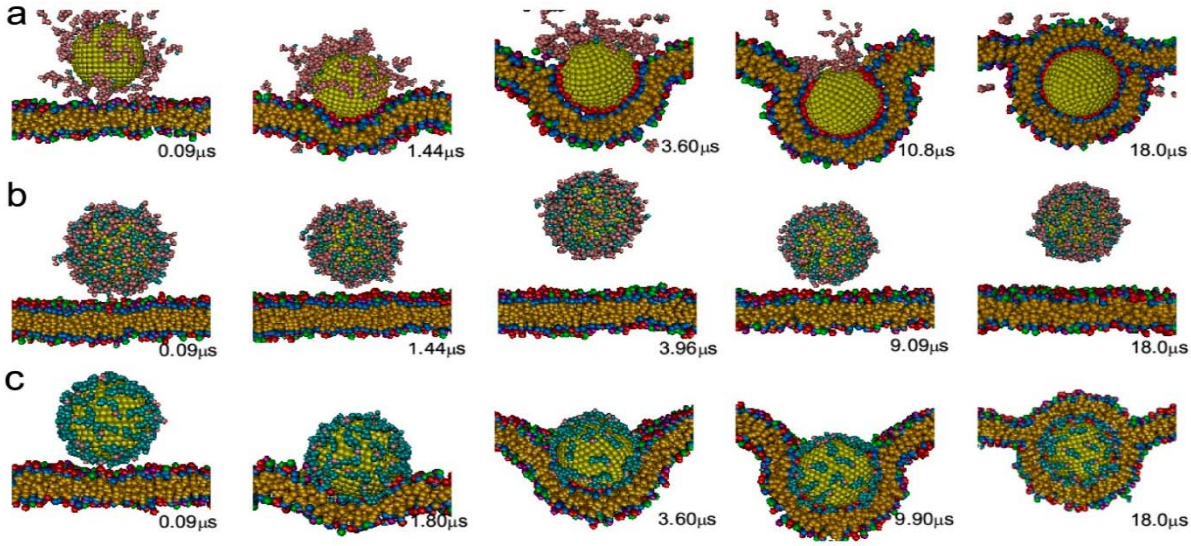


Figure 4: Time evolution of nanoparticle-polymer complex endocytosis as a function of pH.

*[Adapted with permission from Macmillan Publishers Ltd. (19)]*

At low pH ($pH \quad pK_a \quad 0.48$, i.e., $N \quad 3$), due to the less number of ionized monomer, polymer adsorption on the surface is weak, which not hinder the interaction between the receptors on the membrane and ligands on the nanoparticle surface resulting in particle engulfment (Figure 4a). When pH is in the middle range ($pK_a \quad 0.30 \quad pH \quad pK_a + 0.30$, i.e., $4 \quad N \quad 8$), stable adsorption of ionized monomer occurred on the nanoparticle surface, which prevents receptor-ligand interaction & thus NPC will just weakly attach to or get away from the membrane (Figure 4b). When pH is high ($pH \quad pK_a + 0.48$, i.e., $N \quad 9$), strong absorption occurs due to the presence of a high number of ionized monomers, despite this strong electrostatic repulsion between charged polymer monomers themselves, the number of adsorbed polymers is small (Figure 4c). Therefore polymer can't completely prevent the receptor-ligand interaction membrane and then be engulfed by cell membranes. Such a system can be an ideal carrier for anticancer therapy wherein a typical cancer cell environment has acidic pH (about 6.5) than a normal cell. This could result in selective high uptake in a cancerous cell with the minimum off-target delivery.

### *b) Endosomal Escape Mechanism of pH-Responsive Gene Delivery Vectors*

Tian and Ma investigated responsive G4-PAMAM dendrimers interacting with symmetric and asymmetric negatively charged bilayers, with and without tension, at both neutral and low pH, to get insights into the gene escape mechanism using coarse-grained (CG) MD simulations –(20). It was shown that both membrane tension and electrostatic interactions between charged dendrimers and tensed membrane play important role in dendrimer penetration through the membrane. As depicted in Figure 5, in the extracellular environment, primary amines of dendrimers are protonated leading to terminally charged dendrimers, which favours endocytosis through the positively charged membrane. In endosomes (pH ~ 5), dendrimers are protonated, leading to increased osmotic pressure and dendrimer swelling, putting the membrane under tension. Meanwhile, electrostatic interactions between charged dendrimers and asymmetric negatively charged endosomal membrane cause a drop in the critical membrane tension required for membrane disruption, allowing nanoparticles to escape from endosomes.

### Challenges and limitation

To deal with the challenges of time constraint, the increase of computational efficiency and speed is

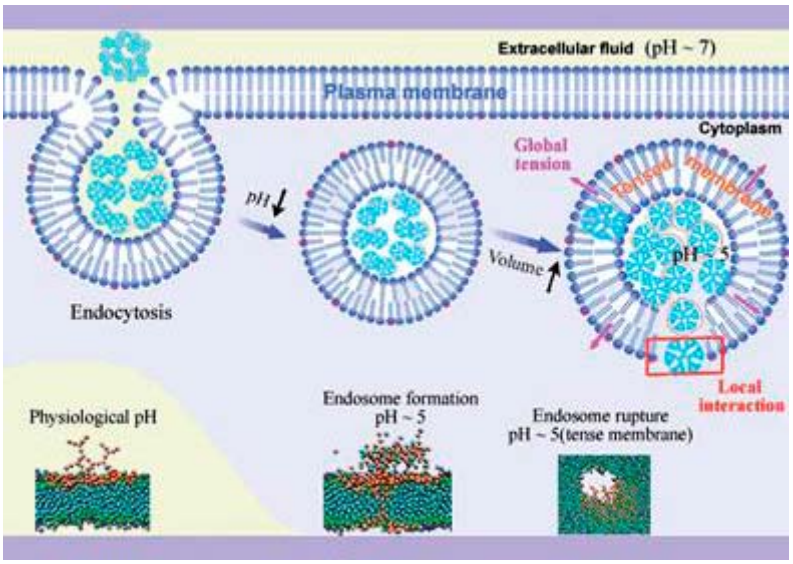


Figure 5: A sketched representation of the escape mechanism of pH-responsive dendrimer for gene delivery.
*[Adapted with permission from The Royal Society of Chemistry (20)]*

crucially important. To reduce the time, a common technique is to use a low-fidelity model to approximate the true model, though strictly speaking there is no such thing as true models because all models are approximations to the reality. However, for practical applications, most computationally extensive models can be approximated by computationally cheaper versions. An important issue is an accuracy that the approximate model can achieve. Typically, high-fidelity models tend to be computationally expensive, while low-fidelity models can speed up and thus reduce the overall computational costs. However, there is always a trade-off between the accuracy of the approximate models and the computational costs. The main challenge is to know how to construct the computationally efficient and yet sufficiently accurate models practically with ease for implementation, which remains unresolved. With vast computer resources to be harnessed for overnight computing, energy consumption may become another major issue (21).

## Conclusion and future perspectives:

As stated by Karplus, Levitt and Warshel (Nobel Prize: Chemistry, 2013) - *"Today the computer is just as important a tool for chemists as the test tube"*, computer simulations have been proved to a great boon. Although currently, computational studies can characterize important aspects of drug delivery systems, a major challenge for the near future is to go beyond individual drug carriers and to investigate at a mechanistic level the larger-scale processes. With advances in computational capabilities, the integrated computational methods will accelerate not only drug development but also help to identify effective formulation strategies. Similar to the crucial role played by computer aided drug design in drug discovery and design, Computational pharmaceutics also has great potential to shift the paradigm of drug delivery research in the near future.

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## Fill in the Blanks

1. _____ is a process or technique by which thin coating can be applied to small particles of solids, droplets of liquids, or dispersion, thus forming microcapsules.
2. In radiation sterilization, there are two types of sterilization techniques, namely sterilization by _____ and _____.
3. Cleanrooms have _____ filters to maintain low particulate count.
4. _____ is the international clinical trial launched by the WHO & partners to help find an effective treatment for COVID-19.
5. The first company to market favipiravir tablets in India under the brand name FabiFlu was _____.
6. In December 2020, Novartis received approval for _____, which is the first and only approved small-interfering RNA (siRNA) low-density lipoprotein cholesterol (LDL-C) lowering treatment in Europe.
7. _____, originally written in Italian, was the first official pharmacopeia, which became the legal standard for the city-state of Florence in 1498.
8. The letter N in N-95 masks stands for _____.
9. _____ was the first research center in Europe to isolate the genomic sequence for SARS- CoV-2.
10. The full form of ICMR is _____.

# Ionic liquids in drug delivery: An overview

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## Introduction

Most of the pharmaceutical drug molecules (approximately 40%) discovered using combinatorial chemistry and 70% marketed drugs in the pharmaceutical industry are plagued with issues of low aqueous solubility, bioavailability, stability and undesirable polymorphic conversion (1). To address the solubility issues, various strategies have been tailored namely, salt-formation, crystal engineering, prodrug, micellar solubilization, complex formation, solid dispersions, and modulation of solubility using lipid/polymeric/lipid-polymeric constructs (2). Since last decade, ionic liquids (ILs) have emerged as solvent of choice owing to their environmentally benign nature, tunability, and multifunctional applications in drug delivery systems. Broadly, ionic liquid can be considered to be molten organic salts comprising large ionic components i.e. organic cations and organic/inorganic

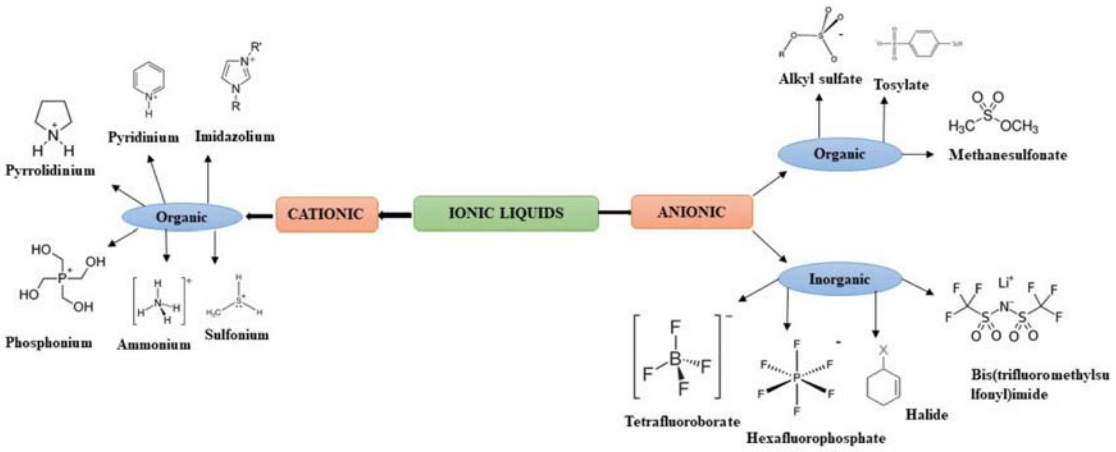


Figure 1: Commonly used cations and anions in ILs (1)

anions (Figure 1), which exhibit unusually low melting points or glass transition temperature i.e. below 100 °C (3).

The low melting point can be ascribed to the presence of large dimensions with conformational flexibility of their ions contributing to small order lattice enthalpies alongwith large order entropy changes, thermodynamically favouring liquid state (4). Typically, at relatively ambient conditions, ILs have unique features such as negligible vapor pressure or non-volatility, very low viscosity, tunable polarity, hydrophobicity, solvent miscibility, acidity, basicity, long-range thermal stability, and very low mineral acid/ base corrosivity (Table 1).

The judicious choice of cations-anions combinations in ILs having balanced ion-ion interactions and symmetry within a wide window of ion structure can result in ILs based solvents/cosolvents/materials with potential to modulate the pharmacokinetic and pharmacodynamic properties of drugs (7).

**Table 1:** Physical properties of Ionic Liquids (5,6)

| Physical Properties | Observed attributes of ionic liquids |
|---|---|
| Liquid range | As compared to molecular solvents, ILs have broad liquid temperature range between its melting point or glass transition point and its boiling point or thermal decomposition. |
| Melting point | ILs have melting point below $100^0$C. Increase in size of anions and cations decreases the melting point. |
| Vapour Pressure | Due to strong coulombic interactions between ions the ILs have negligible vapour pressure. |
| Solubility | ILs can dissolve both ionic and covalent compounds depending upon the polarity and co-ordination ability of ions. i.e. they can dissolve both polar and non-polar solutes. |
| Viscosity | ILs are more viscous than molecular solvents. The viscosity lies in the range of 10-500cp. Increase in chain length increases the viscosity. Weak coordinating anions have lower viscosity than strong coordinating anions. Increase in temperature causes corresponding decrease in viscosity. |
| Density | Density of ILs is shown to exceed that of water, generally in the range of 1-1.6 $gcm^{-3}$. Increase in bulkiness of cation and anion decreases the density of ILs. Increase in temperature has no effect on density of ILs. |
| Surface Tension | The surface tension of ILs is lower than molten inorganic salts and water. Temperature has linear effect on surface tension of ILs. Surface tension increases with increase in size of anion. |
| Refractive Index | ILs have high refractive index which is also depend upon the nature of anion and cation. Refractive index of IL increases with increase in branching and alkyl chain length of cation. |
| Conductivity | The presence of large number of cations and anions endows ILs with good specific conductivity. |

## Different types of commonly used ILs in drug delivery

- **Imidazolium based Ils**

Among the different type of ILs, imidazolium based ILs have been widely studied. The type of nitrogen atom substituents and the counter-ions can greatly influence the physical properties of Imidazolium ILs viz., melting points, solubility and viscosity. The inorganic anions in imidazolium based ILs is known to decrease the biodegrability of ILs (7). Despite its stability and ease of synthesis, this class is associated with toxicity issues (8). Santos de Almeida et.al. (2017) investigated the cytoxicity, skin permeability and solubility enhancement of Caffeine in imidazolium based ILs [C2-C6mim][Br], and choline-based ILs [Cho][Phe] /[Glu]. The study revealed that unlike choline based ILs, though solubility

and skin permeability of caffeine was improved, cytoxicity also was found to proportionally increase with imidazolium based ILs with increasing alkyl chain length (C6>C4>C2) (10).

- **Choline based Ils**

Choline also known as 2-hydroxyethyltrimethyl ammonium chloride is a very important class of ionic liquid due to its excellent biodegradability and aqueous solubility. In comparison to the imidazolium class of ILs, choline based ILs are found to have lower toxicity. It is reported that biodegradability of ionic liquid is greatly enhanced with increase in the alkyl chain length of cation or by the presence of functional groups such as hydroxyl, carboxylic, alcohol or ether on these alkyl chains or by presence of choline (11). However, toxicity increases with increase in alkyl chain length in anion but decreases with presence of polar functional group on side chains (8). Examples of choline based ILs include choline lauryl sulphate, choline triflate, choline acesulfamates, choline laurate, to name a few which are used as surfactants, solubilizers with low toxicity (12). Tanner et al. (2018) demonstrated the delivery of insulin via transdermal route using choline based ionic liquid . They investigated the effect of varying ratio of cation/anion in Choline and Geranic acid (CAGE) based DES on transdermal transport of insulin. CAGE with varying range of 1:4 to 2:1 was found to enhanced the transport of insulin into dermis as compared to geranic acid alone (13). Similarly, Yuan et.al. (2020) showcased the enhanced solubility and permeability of ibuprofen across polyethersulfone membrane using low concentration (0.8 wt%) of two relatively non-toxic cholinium-animo acid based ionic liquids, cholinium glycinate [Ch][Gly] and cholinium analinate [Ch][Ala] (14).

- **Active Pharmaceutical Ingredient ILs (API-ILS)**

Often, active pharmaceutical ingredients (APIs) used in drug formulations are solid crystalline in nature. Although they have better stability, few may have limitations such as poor solubility, permeability, polymorphism. To circumvent these issues, API based ILs have been explored, also known as third generation API-ILS (1). Hough et.al. was the first to synthesize a single active API-IL, ranitidine docusate (Tg -12 °C), which was obtained via interaction of ranitidine hydrochloride and sodium docusate (15). Bica et al. has demonstrated the enhancement of aqueous solubility of aspirin using dual-functional API-ILs containing aspirin and its metabolite, salicylic acid (16). Fernandez-Stefanuto V, Tojo E. reported that the counterions, derived from 2-dimethylaminoethanol (DMEA), tetramethylguanidine (TMG), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,4-diazabicyclo (TED) were effective in increasing the aqueous solubility of two drugs namely indomethacin and mebendazole. It was observed that salt containing [TED][IND] API-ILs increased aqueous solubility about 600 folds and the salt containing [MEB][Gly]API-ILs has shown to increase the water solubility upto 25 folds (17).

## Applications of ILs in drug delivery

Typically, desirable traits of any drug delivery system is that it should facilitate improvement of druggability and bioavailability of drug. For this, the two key determinants recognized are solubility in aqueous milieu and permeability across biological membranes. Ionic liquids have shown to influence these key determinants via complex interactions thereby enhancing the bioavailability of drug (1). Besides this, ILs have been shown to facilitate production of nanoparticles owing to their unique features.

- **ILs as solvent for solubility enhancement**

The solubilizing mechanism of the drugs in ILs is influenced predominantly by the type of anions. The solvation capability of ILs is governed by the number of hydrogen bonds between anion and drug. The contribution of cations is found to have negligible effect on solubility (1). Alawi et.al. (2015) elucidated the mechanism of solubilization of antidiabetic drug, glibencamide in cholin-tryptophan ILs. The study suggested that the drug was found to be localized between the stacks of ILs via formation of multiple hydrogen bonds and – interaction through the aromatic rings between the aromatic sulfonamide and aromatic rings of IL (18). An interesting observation has been reported by Mc Crary et.al. (2013), the study suggested that for solubilization amphiphilic and BCS Class IV molecules, Amphotericin B (Amp B) and Itraconazole, it is desirable to select complementary functionality in either one or both ions constituting an IL excipient to create desired HLB to solubilize drug. In case of Amp B, ILs were designed using the hydrophilic, strongly basic, hydrogen bond acceptor [OAc] anion with an intent to disrupt the inter and intramolecular hydrogen bonding within the hydrophobic section of Amp B. For balancing the HLB, short-chain fatty amines with varying alkyl chain length was used as hydrophobic cations. As the alkyl chain length increased there was corresponding decreased in solubilization of Amp B observed; plausible due to increased interchain interactions within the fatty amines. Hence, it is essential to understand the chemistry of drug and ILs before designing ILs based drug delivery system (19). Recently,

Sintra et.al. (2018) elucidated the role of ILs as hydrotropes in enhancing aqueous solubility of ibuprofen by the formation of IL-ibuprofen aggregates. In this study, the authors observed that both cations and anions contributed to the hydrotropic mechanism of solubilization. Two series of catanionic hydrotropes were studied; chloride-based IL cation series, wherein non-cyclic, non-aromatic [N4,4,4,4]Cl and [P4,4,4,4]Cl demonstrated enhancement in aqueous solubility of drug. Similarly, IL anion series of 1- butyl-3-methylimidazolium family, dicyanamide and thiocyanate anions exhibited superior hydrotropic solubilizing capability (20).

- **ILs as solvent for permeation enhancement**

Broadly, the mechanism of permeation enhancement using ILs has been ascribed to i. electronic profile of ionic liquids, ii. membrane fluidization mostly within protein and lipid regions promoting paracellular transport , iii. partitioning into the epithelial membrane by creating channels for transcellular transport (21). Zheng et.al. (2020) demonstrated enhanced skin permeation enhancement of model drugs,5-Fluorouracil (5-Fu) and Hydrocortisone (HC) using ILs (L-proline dodecyl ester hydrochloride and L-leucine dodecyl ester hydrochloride). They concluded that the enhanced permeation of drug was ascribed to lipid fluidization and lipid extraction occurring due to the interaction of ionic liquid with the intercellular lipid domains (22). Zhang et.al. (2017) demonstrated the similar skin permeation enhancement of model drug, testosterone using imidazolium ILs . The study revealed that the ILs changed the stratum coreneum surface properties by disrupting the the corneocytes arrangement, rendering skin more permeable (23). Hattori et.al. (2019) has demonstrated the safety of ionic delivery in comparison to ethanol for the transdermal delivery of Nobiletin. The study revealed that unlike ethanol which showed nonselective delivery of Nobiletin and skin irritation due to gross conformational alterations in the stratum corneum proteins, ionic liquid comprising of choline and geranic acid only affected the skin lipid enabling deeper skin permeations (24).

- **ILs as solvent for production of nanoparticles**

It has been reported that the low surface tension of ILs enables faster nucleation rate which can benefit generation of smaller particulate system. Further, the electronic property can confer electronic and steric stabilization of nanoparticles. In addition the ability of ILs to form strong hydrogen bonded networks aids in controlling shape characteristics of the synthesized nanoparticles (25). Julio et.al. (2019) have shown the combined utility of choline-based ILs and poly (lactic-co-glycolic acid) to formulate rutin loaded nanocarriers (250–300 nm and a zeta potential of approximately 40 mV) having 51% to 76% drug association efficiency of rutin. The resultant nanocarriers exhibited sustained release profile showing 85% of rutin released after 72 h (26). Recently, Lu et.al explored amphiphilic block copolymers using hydrophobic polyionicliquid block which was shown to enhanced stability of light and pH sensitive doxorubicin loaded nanoparticles for enhanced tumor growth inhibitor activity (27). Ali et. al. (2020) has also demonstrated the use of ILs ([C1 mim][(MeO)2PO2, cholinium oleate, cholinium linoleate, and cholinium erucate), along with sorbitan laurate, and isopropyl myristate to form IL/Isopropyl myristate microemulsion having droplet size of 6.5 to 21.2 nm with almost 4.7- and 5-times higher loadings of Celecoxib and acyclovir, respectively (28).

## Conclusion

Herein, we have provided a brief overview about the potential applications of ILs in drug delivery. Owing to the tunable properties, ILs can contribute in both enhancement of the drug solubility and the permeability as well as confer stability for those drugs having polymorphic issues. In addition, ILs are now being increasingly explore in fabrication of drug nanocarriers due to ecofriendly and myriad of structural advantages. Although, not discussed, there is research being undertaken to explore the potential of these green solvents in overcoming multidrug resistance in cancer and antimicrobial domains. However, there is much yet to be explored about their mechanism, the biodegradability and toxicity. Nevertheless, these systems offer huge opportunities for creating new delivery approaches for administering drugs via. oral, pulmonary, ocular, parenteral routes.

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# CRS IC NEWS

- Highlights of CRSIC 2020
- Achievements of 2020-21
  *Reaching New Heights*

# Glimpses of the 18th International Symposium

on

## "ADVANCES IN TECHNOLOGY AND BUSINESS POTENTIAL OF NEW DRUG DELIVERY SYSTEMS"

held on 28th and 29th February 2020 at Hotel Sahara Star, Mumbai, India.


*Lamp Lighting at the inaugural session by Dr. Clive Wilson, Chief Guest and Mr. Ajit Singh Chairman ACG along with the Executive Committee Members of CRSIC*


*Dr. V. G. Somani – Drugs Controller General of India being felicitated as Guest of Honor by Mr.Ajit Singh, Chairman ACG and Dr. Parizad Elchidana, President CRS IC.*


*Release of 11th Edition of CRS IC Newsletter by Dr. Clive Wilson, Chief Guest at the Symposium.*


*Eminent speakers at the 18th International Symposium along with the Executive Committee Members of CRS IC*

# Achievements of 2020-21

*Reaching New Heights*

## National Webinar on "Vaccine Manufacturing: Opportunities and Challenges"

CRS IC organized its very first National webinar titled "Vaccine Manufacturing: Opportunities and Challenges" on 2nd May 2020. Dr. S. S. Pisal, currently working as a Director, R&D & Manufacturing at Serum Institute of India, Pvt. Ltd., Pune was the invited speaker for this webinar.

He delivered an insightful and enlightening talk sharing the long standing experience in the field of vaccine formulation development, upstream/downstream processing of vaccines. He explained the intricate details of vaccine development focusing on lyophilization process, one of the most crucial steps during vaccine manufacturing. The national webinar was well received by more than 100 participants.

## National Webinar on "Oral Delivery of Biologics"

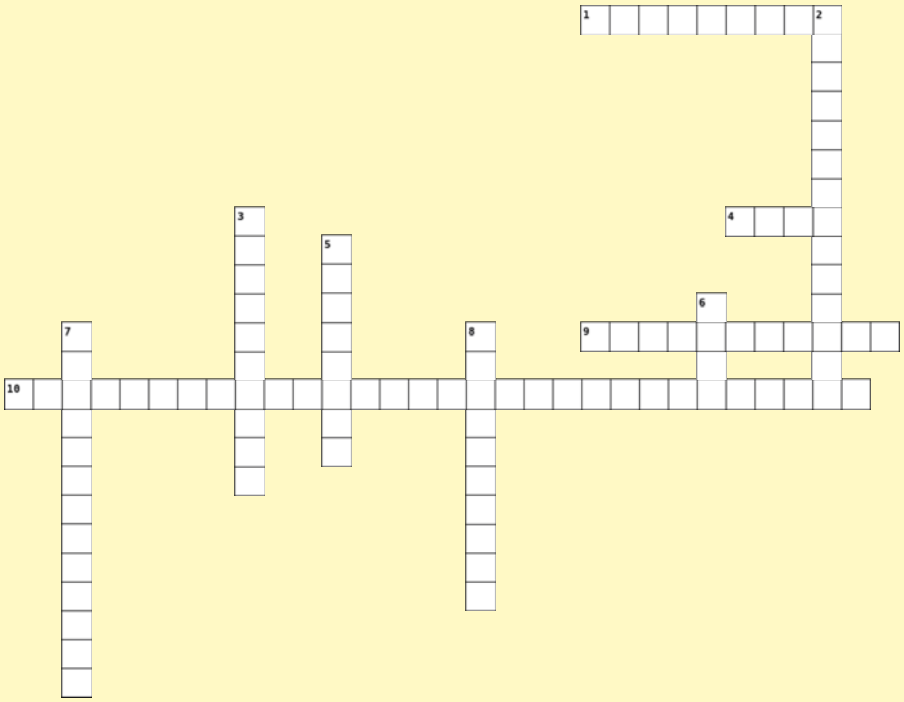
CRS IC conducted the second National webinar titled "Oral Delivery of Biologics" on 29th Aug 2020. Dr. Yogeshwar Bacchav, Founder Director, Adex Pharma & Co-opted member of Executive Committee, CRS IC was the invited resource person for this technical talk. Oral delivery of biologics like proteins, peptides, and hormones especially insulin has always been a topic of great interest and extensive research. Dr. Yogeshwar presented the challenges in the oral delivery of biologics and went on to explain the formulation strategies to overcome these challenges. His lucid and informative talk acquainted the participants with the latest nanocarriers and advanced medical devices explored for the effective oral delivery of the biologics.

## National Workshop on "Manufacturing, Characterization, and Applications of Monoclonal Antibodies"

CRS IC conducted a national level workshop on the topic "Manufacturing, Characterization, and Applications of Monoclonal Antibodies" on 10th October 2020. The workshop was designed to provide insight into the development, manufacturing, and applications of monoclonal antibody-based formulations. The event received an overwhelming response from the students, academicians, and industry personnel with about 800 registrations from across India, Ireland, UK, US, Germany, and UAE. On the occasion of the workshop, the upgraded website of CRS IC was launched in the gracious presence of Mr. Ajit Singh, Chairman, ACG World.

Dr. K. Rajeshwari, Founder and Managing Director of Bioklone Biotech Private Limited, Chennai, delivered the first session on "Development of human monoclonal antibodies". She briefed about the emergence and evolution of antibody platforms followed by the major challenges and alternate strategies involved in the development of human monoclonal antibodies. Dr. Suneet Shukla, a Senior Pharmacologist at FDA Washington D.C. Metro Area, delivered the next talk on "Basics of Monoclonal Antibodies Drug Development". His talk focused on the characterization of monoclonal antibodies as drugs and important aspects of preclinical and clinical development of antibody drug development. The next speaker was Dr. Sachin Dubey, Deputy Director & Head of the Formulation, Analytical & Drug Product Development, Ichnos Sciences, Switzerland, who presented a talk on the topic "Role of antibodies based therapeutics in the modern healthcare system". He highlighted the impact of monoclonal antibody in the management of oncology/ autoimmune diseases, the development of antibody-drug conjugates, and the possible applications of antibodies outside therapeutics, for instance, in diagnostics, purification etc.

## Pharma-Wiz



**Across::**

1.designed to release a drug at a predetermined rate

4.any substance that causes a change in an organism's physiology

9.the extent and rate of solution formation from a dosage form

10.A substance used in a finished pharmaceutical product

**Down::**

2.the breaking down of something into small particles

3.being in accordance with the regulations set out by regulators

5.drugs that have few or no pharmacological effects by themselves

6.specified amount of medication taken at one time

7.electrokinetic potential in colloidal systems

8.the tendency of a solid substance to break into smaller pieces

## WORD SEARCH

| Q | Z | W | V | A | C | C | I | N | E | Y | B | K | G | K | D | Y | G | K | I |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| F | N | C | J | T | C | H | D | S | S | E | S | W | L | I | E | D | M | D | F |
| C | S | O | C | I | V | E | J | K | V | S | K | Y | L | C | B | U | Y | K | E |
| O | Y | R | G | V | Y | A | S | X | D | P | F | A | V | B | N | G | B | F | C |
| N | S | O | O | I | E | L | G | T | H | I | T | G | L | I | I | J | D | K | T |
| T | Q | N | V | R | I | T | D | G | J | R | P | H | T | R | D | Q | U | D | I |
| A | H | A | F | A | D | H | O | I | O | A | T | Y | A | E | E | R | A | XV | O |
| G | J | G | R | L | A | C | K | M | W | T | K | O | P | L | T | O | V | H | N |
| I | E | T | A | S | D | A | F | D | U | O | H | O | S | D | E | G | S | E | F |
| O | E | K | C | E | G | R | N | S | H | R | D | G | H | W | C | L | U | B | R |
| U | Q | V | N | K | B | E | I | Y | F | Y | B | J | K | K | T | V | O | P | E |
| S | H | I | R | M | R | G | P | S | B | T | M | E | R | S | I | H | N | D | P |
| P | K | R | U | R | C | C | L | Q | K | U | U | D | A | Q | O | J | E | G | U |
| H | R | U | D | F | N | K | R | E | P | U | T | P | O | S | N | N | V | W | R |
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| T | R | A | N | S | M | I | S | S | I | O | N | G | C | C | O | V | I | D | L |

**List of words:**

ANTIBODIES
ANTIVIRAL
CONTAGIOUS
CORONA
VIRUS
COVID
DETECTION
HEALTHCARE
IMMUNITY
INFECTION
INTRAVENOUS
MORTALITY
MUTATION
PANDEMIC
REPURPOSE
RESPIRATORY
TRANSMISSION
TRIALS
VACCINE

# Hall of Fame

## Awards and Grants

- Ø Dr. Sandhya Shenoy, Associate Vice President, FDC Ltd. received the Healthcare Leadership Award- By ET Now at the World Health and Wellness Congress, February 2020. She also received the Green Belt Certification on Six Sigma Methodology by Indian Statistical Institute, December 2020.
- Ø Dr. Vandana Patravale, Professor of Pharmaceutics, Institute of Chemical Technology, Mumbai received two grants from DBT-BIRAC COVID-19 Research Consortium to develop a simple colorimetric diagnostic technique for Novel Corona Virus Detection and an intranasal mucosal vaccine for COVID-19.
- Ø Dr. Subham Banerjee, Assistant Professor, Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER)-Guwahati, was inducted as the Associate Fellow of West Bengal Academy of Science & Technology (WAST)-2020 and the Member of National Academy of Sciences (MNASc.)-2020, Prayagraj (Allahabad).
- Ø Dr. Subham Banerjee was invited as an Evaluator of Toycathon-2021, at the Ministry of Education's Innovation Cell, Govt. of India. He also won the First Prize (2021), at the BRTC Mapping the Change makers of North East Region of India, Govt. of India. He is also the recipient of the Best Poster Award (Co-author) (2020), at the Dissolution Discussion Meeting, Agilent Technologies, USA and the Selected Front Cover Page (2020) in ASSAY & Drug Dev. Tech., Mary Ann Liebert Inc., New York, USA.
- Ø Dr. Subham Banerjee also received the Best Technology Award (2019-20), and the Best External Cash Flow Award (2019-20), at the 12$^{th}$ Foundation Day of NIPER-Guwahati, Assam.
- Ø Dr. Sanjay B. Patil, Associate Professor, SNJB's Shriman Sureshdada Jain College of Pharmacy, Chandwad, Nashik received the Grant-in-Aid of INR 15 lakhs under the scheme "Modernization and Removal of Obsolescence Rural (MODROB-Rural)" from All India Council for Technical Education (AICTE), New Delhi.
- Ø Dr. Ashlesha P. Pandit, Professor, JSPM's Rajarshi Shahu College of Pharmacy & Research, Pune, received Dr. P. D. Sethi Memorial Annual National Awards 2019 for 'Best Research Papers on Application of TLC/HP-TLC in Pharma, Herbal & Other Chemical Analysis' for the paper titled 'Curcumin as a permeability enhancer enhanced the antihyperlipidemic activity of dietary green tea extract' published in BMC Complementary and Alternative Medicine, vol. 19, pp. 129-139.
- Ø Dr. Mangesh D. Godbole, Assistant Professor, Kamla Nehru College of Pharmacy, Nagpur won the consolation prize for the poster presentation for the poster titled 'Formulation and development of thermo responsive in situ gel of atenolol for nasal delivery' at Society for Research Development in Health Sciences (RDHS) sponsored, 2nd International conference on Invigorating research in pharmaceuticals: Reasonable industrial approach, organized by Dadasaheb Balpande College of Pharmacy Besa Nagpur, 14-15th February 2020.

## Patents

- Ø Dr. Vandana Patravale, Professor of Pharmaceutics, Institute of Chemical Technology, Mumbai was granted an Indian patent for "Highly Porous Tablet" along with Ammar M Arsiwala, Dalapathi B Gugulothu, and Rashmi H Prabhu (Patent No-341894, Date of Grant: 17 July 2020). She was also granted a patent for "Topical Composition" along with Mehul Shah, Lalatendu Panigrahi, and Pratik Kakade (Application No: 201921019828 a, Date of Publication: 27 November 2020). Another patent on "Vaccine delivery system for non-invasive immunization against brucellosis using green technology" was also granted to Dr. Patravale (Patent No: 358722, Date of Grant: 17 February 2021).
- Ø Dr. Subham Banerjee was granted an Indian Design Patent along with Tushar Kanti Malakar, VGM Naidu, and USN Murty for "A face protecting device" (Application No. 329319-001, Cbr No. 8169, Date of Grant: 14 January 2021), and an Indian Patent for "Medicated skin patch, use and method of making thereof" along with Vishal Sharad Chaudhari, Tushar Kanti Malakar, and USN Murty (Patent Number: 355267, Date of Grant: 05 January 2021).

## Student Awards

- Ø Mr. Shivraj Naik, PhD Research Scholar at Institute of Chemical Technology, Mumbai received the BIRAC SITARE-GYTI Award (Students Innovations for Translation & Advancement of Research Explorations - Gandhian Young Technological Innovation Award) consisting of a Certificate, Memento and research grant of Rs. 15 lakhs.
- Ø Mr. Sagar B Dhoble, PhD Research scholar at Institute of Chemical Technology, Mumbai received first prize of USD 500 in a virtual international event MIT COVID 19 Challenge – India in the category - Ensuring safety and well-being of frontline workforce and healthcare practitioners. He also participated in the COVID TechConnect Innovation Spotlight Summit and was adjudged among the 15 Groundbreaking Innovators in SARS-CoV-2 detection for his idea "A simple colorimetric onsite COVID 19 detection kit from environmental samples".
- Ø Ms. Sony Priyanka, PhD Research Scholar, BITS Pilani, Hyderabad received the gold medal from Jawaharlal Nehru Technological University, Hyderabad (JNTUH) for being the University topper in Master of Pharmacy. She also received the DST-INSPIRE fellowship to pursue doctoral studies.
- Ø Ms. Priya Sharma, currently pursuing Ph.D. from Amity University, Noida was associated with the first Pumas Boot camp and the release of a pharmacometric learning repository from India that was built from ground up using Pumas (Pharmaceutical Modeling and Simulation) platform. She was also the North zone first prize winner and a finalist in the Society for Pharmaceutical Dissolution Sciences (SPDS) webinar conducted in collaboration with Association of Pharmaceuticals Teachers of India(APTI), India., Jun 2020.
- Ø Ms. Priyanka Salunkhe and Mr. Vaibhav Ghegade, Postgraduate students at Institute of Chemical Technology, Mumbai were selected to participate in Novartis India's BioCamp 2020-21 from among 1800 applicants across the country.

# Answer Key

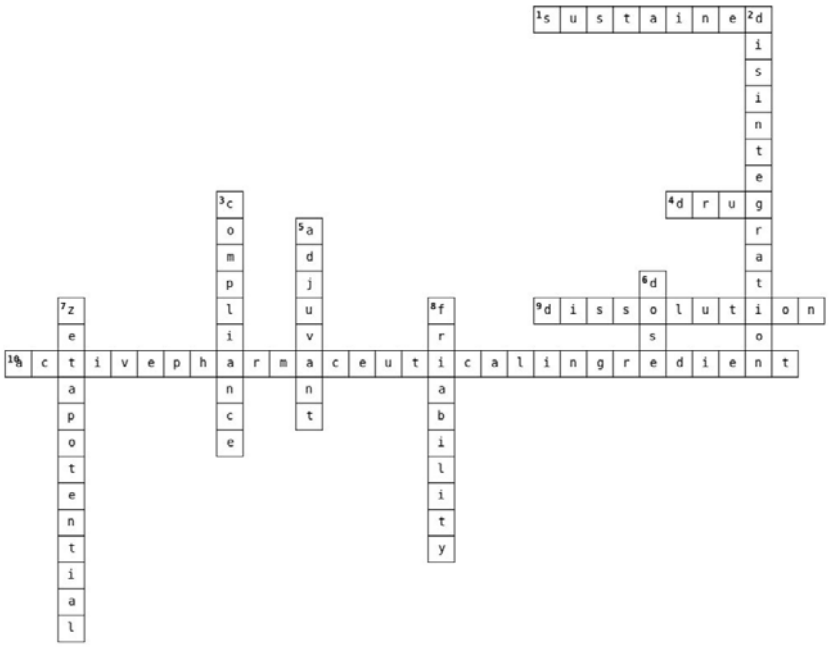
## Answers for MCQ:

1. a) 120 g
2. c) Brazil
3. b) China
4. a) Flow-Through Cell
5. b) Serum Institute of India Ltd.
6. c) Microcrystalline Cellulose
7. b) Prednisolone tablets
8. b) CRISPR-Cas9
9. c) Lepromin test
10. a) The number 40

## Answers for Fill in the Blanks:

1. Microencapsulation
2. UV; Ionizing Radiation
3. HEPA
4. SOLIDARITY
5. Glenmark Pharmaceuticals Ltd.
6. Leqvio (inclisiran)
7. *Nuovo Receptario*
8. Not resistant to oil
9. Lazzaro Spallanzani Institute
10. Indian Council for Medical Research

## Pharma-Wiz



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1. designed to release a drug at a predetermined rate
4. any substance that causes a change in an organism's physiology
9. the extent and rate of solution formation from a dosage form
10. A substance used in a finished pharmaceutical product

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3. being in accordance with the regulations set out by regulators
5. drugs that have few or no pharmacological effects by themselves
6. specified amount of medication taken at one time
7. electrokinetic potential in colloidal systems
8. the tendency of a solid substance to break into smaller pieces

## Word Search

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