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Review Article

ALTERNATIVES FOR REFRIGERATED VACCINES: A CONTEMPORARY PREREQUISITE

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ABSTRACT

Immunological preparations like vaccines are vital for the progress of global population. For vaccines to be effective, it is essential that they are stored and preserved under refrigeration. This calls for infallible cold chain supply in order to maintain the potency of vaccines from manufacturer to user. Enormous investments are required in the development and maintenance of cold chain logistics and related infrastructure which in turn elevates the cost of immunization. Such expensive vaccines and other related issues of apprehension disappoint the purpose of preventive healthcare. Hence, several technologies are sprouting out as alternatives for refrigerated vaccines. This review highlights the recent developments, existing R&D status and future scope in vaccine technology.

Keywords: Cryoprotectants, Adjuvants, HydRIS.

INTRODUCTION

Immunization is an inherent component of several healthcare programs. It is the most favorable approach of protecting the individuals and communities from infectious diseases. Every year, more than 2.5 million young lives are shielded from various dreadful infections owing to immunization [1]. Still lives of millions of infants and human beings are at risk without vaccines. Resistance development, expensive vaccines, supply and storage of vaccines are the key topics of concern.

Vaccines are sensitive biological substances that can either lose their potency and effectiveness or undergo mutations if they are exposed to heat, light or extreme climatic conditions [2]. Hence, most of the vaccines are either lyophilized or maintained in cold storage. Nevertheless, freezing damages most of the vaccines by causing mechanical fractures. Even the cryoprotectants may generate excipient-incompatibility and toxicity issues (Figure 1). Exposed vaccines can result in a reduced immune response and associated adverse reactions. Principally, the loss of vaccine potency cannot be reversed. Major costs in developing vaccines are their maintenance and storage using cold chain supply [3]. Manufacturers have to be sure that vaccines are refrigerated all the way from the production plant to the end-user, whether they are in the western world or in the remotest village in Africa [4]. Evolution of an alternative storage facility for vaccines at room temperature would reduce the cost of immunization without compromising over their safety and efficacy.

Globally, scientists are striving to deal with one of the world's worst problems in healthcare - defective and inadequate cold chain for vaccine storage. There is a vital need for simple and economical vaccines, stable even at tropical temperatures without the need for freezers and associated health-care infrastructure. Such vaccination efforts would revolutionize the healthcare picture, particularly in the developing nations like Africa and India where infectious diseases kill millions of people each year. This article will review recent, analogous efforts of creating thermally stable, ready-to-inject, contamination-free forms of vaccines.

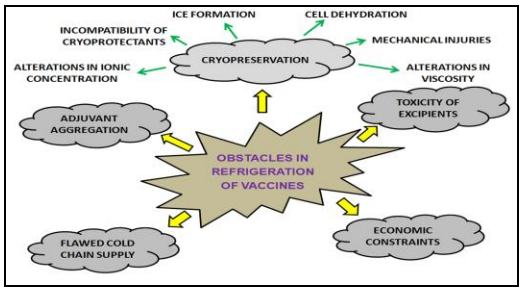

Fig. 1: Obstacles in refrigeration of vaccines

Cryopreservation and related substrates

Biological preparations, microbial cultures or immunological products were conventionally preserved via continual sub-culturing, storage under mineral oil, etc [5]. Generally these techniques are cost-effective but time consuming, laborious and impractical from commercial point of view. In addition, they fail to sustain long-term storage protocols. So, cryopreservation technique was introduced in which temperature was maintained from -60°C to -130°C in ultrafreezers or at -196°C with liquid nitrogen. This technique was well accepted as the best way to maintain cell viability and shelf life for all viable products of biological origin [6,7]. Still preservation at -196°C often necessitates the substantial initial expenditure which would add-on to the price of immunization. A low cost alternative to this would be cryopreservation at -20°C. Yet such freezing temperatures would prove to be a challenge for cell cryopreservation as they often result in cryofractures due to ice formation, water migration and ion concentration (Figure 1). Ice formation in cell interstices, one of the major cell injuries during cryopreservation (-20°C) result into mechanical injuries [5,8]. In order to overcome such cryofractures, cryoprotectants are used which can decrease ice formation and modify membrane elasticity so that the probability of cellular breakage decreases. Even freezing of external water at -20°C leads to cell dehydration with increase in ion concentration [9]. Subsequently, freezing temperature of the cell-cytoplasm decreases but viscosity and concentration of mixture increases. This would affect the cell-metabolism which would end in creating irreversible damage to cellular contents.

Recently, Colauto et al. reported the encouraging effects of substrate and cryoprotectant combination on the cell viability of cryopreserved culture even after confronting with number of freeze-thaw cycles [5]. Their findings suggested that wheat grain like substrates combined with glucose or saccharose were effective in maintaining the viable nature of microbial culture, post cryopreservation at -20°C, up to 3 years. Such substrates contain carbohydrates and proteins that bind with water effectively. This would reduce free water content thus, preventing intercellular crystal formation and cell-dehydration. Semi-permeable cryoprotectants, saccharose and glucose are best cryoprotectants for long term cryopreservation at -20°C and -70°C. Their mechanism of action includes linking free interstitial water and accelerating cell dehydration so that ice formation and mechanical injuries are averted. Vaccines like preparations are known to embed easily within porous, biodegradable materials [10]. Also, dimethyl sulfoxide (DMSO) and glycerol, known to penetrate the cellular wall and the plasma membrane help to prevent cryofractures effectively [11] but their associated toxicity profile overshadows their cryopreservation applications.

Freeze-stable vaccine formulations containing adjuvants

USFDA has approved only aluminum salts like aluminum hydroxide, aluminum phosphate and alum as adjuvants in immunological preparations for human consumption [12]. Aluminum salt adjuvants tend to agglomerate when subjected to freezing and thawing cycles (Figure 1), thereby leading to loss of vaccine potency. During cold chain storage and supply, vaccines with adjuvants undergo multiple freeze-thaw cycles wherein they get exposed to sub-zero temperatures for hours together which would diminish the efficacy of vaccines.

Braun et al. introduced economical GRAS excipients, glycerin, polyethylene glycol (PEG) 300, and propylene glycol (known for their protectant properties against aggregation of vaccine particles) to a specific vaccine preparation formulated with an aluminum hydroxide adjuvant [13]. Outcome of their study depicted that the said protectants at 50% concentration prevented the agglomeration of vaccine particles without compromising their antigenicity. Protectants at higher concentration inhibit the freezing of the vaccine at -20°C by lowering the thermodynamic freezing point without affecting their immunological profile. Moreover, PEG 300 and propylene glycol give rise to vaccine formulations with fluorescence which aid in detecting molecular alterations with thermal fluctuations. Concentration of the protectant must be optimized enough to prevent vaccine from freezing, agglomeration, protein denaturation and loss of immunogenicity. Besides it is essential to maintain the amount of protectant as minimum as possible with the intention of retaining the osmolality [14] and hence, the cost of finished product. Long term stability protocols and sophisticated analytical techniques are insisted upon to assess the preservation of antigen structure and immunogenicity. Additionally, safety and compatibility of excipients, adjuvants and vaccine formulations need to be addressed for stability purpose.

Thermostable Liquid Vaccines

Cryopreserved vaccines can also eliminate the cold-chain. But they still need to be reconstituted before use. Additionally, vaccines are often destroyed by the addition of contaminated water/dilution fluids and that too in erroneous quantity [15]. Recently, thermostable liquid vaccines have been developed successfully that can address such challenges by creating ready-to-inject vaccines [16]. They offer an additional advantage owing to the inert nature of the carrier by means of which more vaccines could be packed into one injection. These new stable liquid vaccines carry the potential for 'modified release' which would reduce the need and cost for booster doses. Recently, this ideology has been transformed into Hypodermic Rehydration Injection System (HydRIS) owing to the collaborative efforts of scientists at the Cambridge Biostability Ltd., the Jenner Institute and Nova Bio-Pharma Technologies Ltd., UK. Alcock et al. evaluated this complete concrete technology for effectiveness, viability and stability of vaccines [17]. HydRIS technology is based on the ability of the disaccharides like trehalose and sucrose to form a glass. This glass is an infinitely viscous anhydrous liquid, functionally a solid, in which molecules are immobilized without any chemical interaction. This phenomenon is derived from the ability of anhydrobiotic organisms to survive desiccation [18,19,20]. Due to this property, non-reducing sugars are commonly used as cryoprotectants and excipients in spray-dried or lyophilized formulations of biological origin.

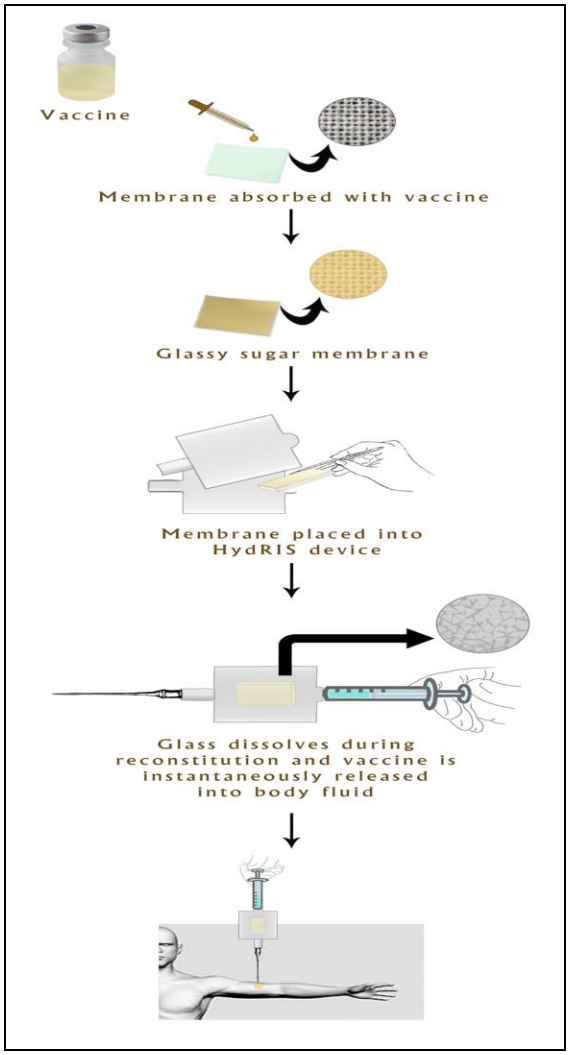

Fig. 2: Schematic representation of HydRIS concept, All-in-One Ready-to-Inject vaccine delivery device [17]

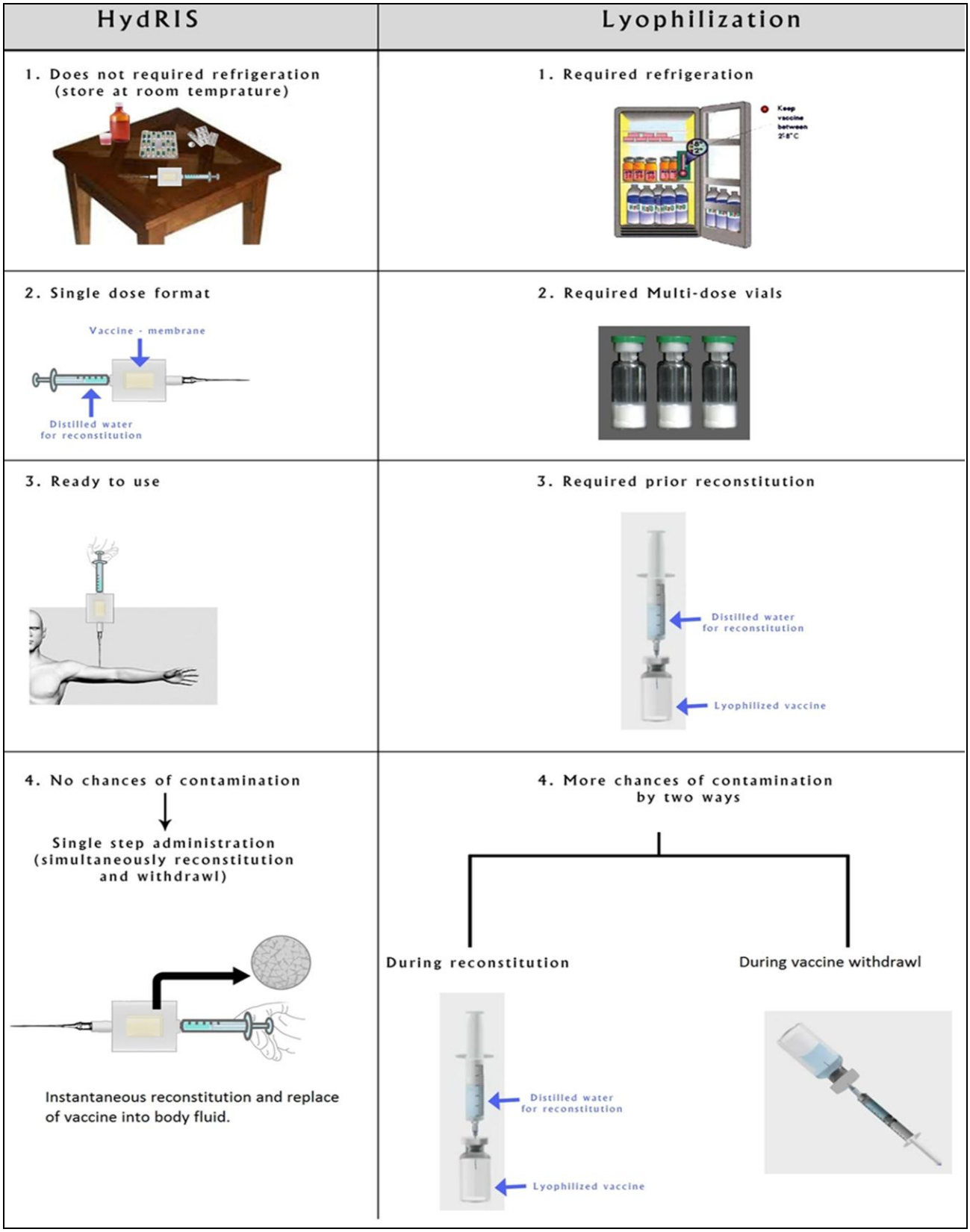

Fig. 3: Comparison between HydRIS technology and Lyophilization [17]

HydRIS Technology

Robert Alcock et al. fabricated trehalose-sucrose glass at ambient temperature on substrates, woven polypropylene (PP) membranes or glass fiber (GF) membranes [17]. Formerly, the vaccine formulations are pipetted out on the membranes. Consequently, the vaccine adsorbed glass–substrate membranes are desiccated overnight at ambient temperature with low relative humidity. Post drying, an ultrathin sugar glass is formed amongst the fibers of the supporting substrate. Surface area of the glass–substrate

membranes outlines the stability, efficacy, viability and safety of adsorbed vaccine preparations. Larger the surface area, more efficient would be the evaporation/drying of glass–substrate membranes. Hydrophilic nature of membrane facilitated instant reconstitution. Crystallinity, glass transition temperature (T_g) and dissolution profile of vaccines adsorbed glass–substrate membranes implied their performance. In addition to this, the conventional, bulky and fragile vaccine vials may get replaced with an aseptically packaged delivery device akin to a syringe.

The dried vaccine coated glass-substrate was placed within an injection molded in-line-membrane holder as a constituent of All-in-One Ready-to-Inject vaccine delivery device (Figure 2), termed as HydRIS, aseptically [21]. At the time of administration, vaccine would be reconstituted with simultaneous dissolution of the sugar-glass by the flow of buffer from the syringe, across the in-line stabilized membrane, into the attached needle. Thus, the need for separate reconstitution step and huge packaging requirements was eliminated with emergence of this ready-to-administer technology (Figure 3). Similar break-through technologies need to be explored for delivering vaccines to the remotest parts of the world effectively, wherein poor resources and faulty distribution network hamper the immunization programs.

Miscellaneous developments in vaccine technology

Currently, nanoparticulate carriers are explored as intelligent vehicles for protein antigens [22]. Vaccine delivery systems based on polymeric nanoparticles are well-known for targeted delivery to dendritic cells; activate antigen-presenting cells with control release of the antigens [23]. Liposomes and parallel novel drug delivery systems possess adjuvant-like properties which may result into synergistic effect [22]. As an option to injectable vaccines, appealing sub-millimeter structures (<1 mm) called microneedles have been created for site-specific delivery of vaccines. Prausnitz et al. developed safe, effective and stable vaccine-microneedle technology against pandemic influenza [24]. All these innovative solutions should be perceived with regard to the stability of vaccines in an attempt to overcome defective cold chain supply and pathetic distribution of vaccines.

CONCLUSION

Research and development of various state-of-the-art strategies for development of heat-stable vaccine products that do not require refrigeration or cold chain supply would decrease the cost of vaccination without compromising their efficacy and stability. Optimum concentration and combination of cryoprotectants, adjuvants, favorable inert substrates, deployment of novel drug delivery systems and designing of ready-to-inject devices would give rise to more robust and affordable vaccines. Adoption of such unique stabilization approaches would help to eliminate the evils of fridges, broken freezers, shortage of fuel, deficient power supply, thereby improving the coverage and economy of immunization programs in developing parts of world.

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