Nano discs recently evolved as a novel tool for studying the membrane associated proteins and serve as an effective drug delivery system. Nano discs constitute disc shaped nanoparticles and can be defined as a membrane system which is synthetic in nature and aids in the study of membrane proteins. It is mainly made of phospholipid bilayer and the water repelling edge is isolated by amphipathic proteins called Membrane Scaffolding Proteins [MSP]. Micelles present in the nano discs mimics the property of the biological membrane proteins. It is a powerful technology that competently delivers the drug components in to the right cells in the right tissues. Membrane scaffold proteins are primarily expressed, purified and characterized and self-assembled to form Nano discs by the process of dialysis using biobeads. Nano discs are proven to be effective in the study of membrane proteins because they can fluidize and counterbalance and also help in reclusion, refinement, biophysical and biochemical studies of them. It also presents a more genuine environment than liposomes, bichelles, amphipols and detergent micelles. Major technological advantages of nano discs include the higher stability and carrier capacity and also the increased feasibility of incorporating both hydrophilic and hydrophobic substances of drug carrier. Thus nano discs serves as an excellent system in its ability to precisely control its composition and provide a nano scale membrane surface for investigating molecular recognition events. This article reviews the emphasis of nanodiscs in studying membrane proteins as well as its effectivity in transforming into a major drug delivery system. An overview of published literatures between 1996 and 2017 was conducted to write the review.

ABSTRACT

Nano discs recently evolved as a novel tool for studying the membrane associated proteins and serve as an effective drug delivery system. Nano discs constitute disc shaped nanoparticles and can be defined as a membrane system which is synthetic in nature and aids in the study of membrane proteins. It is mainly made of phospholipid bilayer and the water repelling edge is isolated by amphipathic proteins called Membrane Scaffolding Proteins [MSP]. Micelles present in the nano discs mimics the property of the biological membrane proteins. It is a powerful technology that competently delivers the drug components in to the right cells in the right tissues. Membrane scaffold proteins are primarily expressed, purified and characterized and self-assembled to form Nano discs by the process of dialysis using biobeads. Nano discs are proven to be effective in the study of membrane proteins because they can fluidize and counterbalance and also help in reclusion, refinement, biophysical and biochemical studies of them. It also presents a more genuine environment than liposomes, bichelles, amphipols and detergent micelles. Major technological advantages of nano discs include the higher stability and carrier capacity and also the increased feasibility of incorporating both hydrophilic and hydrophobic substances of drug carrier. Thus nano discs serves as an excellent system in its ability to precisely control its composition and provide a nano scale membrane surface for investigating molecular recognition events. This article reviews the emphasis of nanodiscs in studying membrane proteins as well as its effectivity in transforming into a major drug delivery system. An overview of published literatures between 1996 and 2017 was conducted to write the review.

Keywords: Membrane Scaffolding Proteins, Phospholipid bilayer, Hydrophobic beads, High density lipoproteins

INTRODUCTION

What is nano drug delivery?

The technique by which drug delivery is achieved has a vital role in its performance. Nanoparticles are solid colloidal dispersions having their size in nanometer range [10-1000 nm] [1]. They have the capacity to transform poorly soluble, poorly absorbable, liable biologically active substances into promising deliverable drugs. Nanoparticles have tremendous potential in effective drug delivery. The drug to be delivered is dissolved, encapsulated, entrapped or incorporated into a nanoparticle matrix system. The most common method is by loading the therapeutic agents within the nano particles [2]. Due to the very small size and miniscule diameter, it can easily penetrate the blood capillaries and reach the target site and effectively deliver the drug in the required therapeutic concentrations [3]. Thus by doing in this way, it reduces drug toxicity as well as side effects and enhances drug efficacy. Nanotechnology has gained publicity these days to overcome the problems of gene and drug delivery [4]. The widespread improvements in technological innovations have begun to change the fundamentals of diagnosis, treatment and prevention of diseases. Such technological innovations are referred to as nano medicines [5] and they have the potential to convert upcoming molecular discoveries from proteomics and genomics into huge beneficiary aspects for the patient [6].

Why nano drug delivery?

The rapid enhancement and progress in nanotechnology during the last years has found out that drug delivery methodologies involving nanoparticles will serve effectively as well as efficiently [7] Even, nano gold particles has found its application in the field of bio pharmaceutics [8]. Nanoparticles have the potential to deliver the therapeutic agents accurately as well as carefully into the target site, whether it is an organ tissue, or even a specific cell and also result in the precise release of the drug at the target site. Because of the colloidal nature and a very small diameter(less than 100 nm), they can penetrate through the capillaries into the internal apparatus of even a single cell. The traditional drug delivery methods such as oral route and injection etc. may also find less applicable due to the following reasons, the most common one being the destruction of the drugs at common doses by the stomach as well as the intestinal juices. Thus it will result in poor or hampered absorption of the drugs into the body and will be non-productive or useless. The new evolution of nano drug delivery can compensate this in a promising way [9]. Nanoparticles are proven to have some additional advantages when compared with micro particles due to increased range of intracellular uptake, relative mobility and availability to large areas of biological targets. The small size, increased rate of solubility customised surface and multi functionality of nanoparticles will open a wider access to create newer applications of biomedicine. Thus the therapeutic as well as pharmacological actions of the drug being administered are enhanced. Moreover the novel properties of nanoparticles provide the possibility to interact with complex cellular functions [10]. Nanoparticles have the ability to mimic biological properties [e.g.: tissue engineering, infection] [11]. The development of huge nano scale interventions will change the scientific landscape in terms of diagnosis, prevention and treatment of illness. Interactions of nano particles with biological environment, therapeutic agent stability, cell signalling mechanisms, cell surface receptor targeting etc. are essential to attain effective drug delivery. Anticancer drugs like paclitaxel, doxamethasone and doxorubicin etc. have been formulated successfully using nanoparticles [12]. One of the most difficult malignancies to detect and treat is the brain cancer. The main problem residing with this is the difficulty in in getting imaging and the passing of therapeutic agents via the blood brain barrier and into the brain. Anticancer drugs like lompramide and doxorubicin bound to nano materials have proven to cross the blood brain barrier and released at therapeutic concentrations in the brain [13]. Nanoparticles used in drug delivery systems, for instance ophthalmic drug delivery are usually less than 100 nano meter and is composed of biodegradable materials like lipids, natural or synthetic polymers etc [14]. Cells take up nanoparticles more effectively than micro molecules and serve as the effective transporter and delivery system. Also, nanoparticles being loaded with antiviral therapeutic agents against human immuno deficiency Virus are found as an emerging as well as promising drug delivery system in the treatment of acquired immuno deficiency syndrome (AIDS) [15]. The various nano
particles used in drug delivery include polymeric biodegradable nano particles, metallic nano particles, ceramic nano particles etc. The complete information regarding the nanoparticles used in drug delivery has been explained in Sandeep S et al. [16]. The experimentation in various micro as well as nano particles for efficient drug delivery has been checked from time immemorial, for instance magnetic microsphere experimentation for delivering drug into the colon region [17, 18]. Nanodiscs serve as an efficient membrane system involved in nano drug delivery. The use of nanodiscs in nano drug delivery is only an evolving or emerging system. Apart from the try-outs being conducted for effectively utilising nanodiscs in nano drug delivery, nanodiscs serve the prominent role in the study of membrane proteins [19].

**Nanodiscs: the new revolution in membrane protein study**

Nanodiscs can be defined as disc shaped (discoidal) particles having a flattened or plaid bilayer composed of phospholipids and the bilayer is being enveloped by proteins (e.g.: apo lipoprotein A1) [20]. The bilayer is arranged in such a way that the hydrophobic end is directed instantly towards the opposite side of the film and the hydrophilic end is directed outwards. Nanodiscs were first found out by Siglar and co-workers [21]. Nanodiscs constitute disc shaped nanoscale particles and can also be defined as a membrane system which is synthetic in nature and aids in the study of membrane proteins [1]. It is made up of phospholipid bilayer and the water repelling edge is isolated by amphipatic proteins called membrane scaffolding proteins [MSP]. They are cropped versions of apo-lipoprotein which cover around a strip of lipid bilayer and form a disc shaped particle or in other words nanodisc. They are arranged in a double belt model. In structure, they are identical to high density lipoproteins [23]. It is these membrane scaffolding proteins or in other words msp's that determine the elongated structure of the nanodisc and it has also been found out that as temperature increases, the particle structure become more rounded or disc shaped [24]. MSPs are also involved in determining the thickness of the hydrophobic layer within the lipid bilayer. There is a chance of some disparity between the protein and phospholipid layer. This can be overcome by self-assembled amphiphilic peptides [26]. Major technological advantages of nanodiscs include the higher stability and carrier capacity and also the increased feasibility of incorporating both hydrophilic and hydrophobic substances of drug carrier [27]. A number of membrane proteins have been involved into nanodiscs. It include metabotropic glutamate receptor [28], parathyroid hormone 1 receptor [29], bacterial chemoreceptors [30], cholester toxin [31] etc. Nanodiscs are found in nature as HDL [32]. These lipid protein particles flow through blood and act as a vehicle for carrying cholesterol to liver for downgrading. Primarily they are disc shaped in original form and on interolation with cholesteryl esters in the phospholipid bilayer, they assume a sphere like form [33]. The size of a nanodisc is dependent on the length of modified apo A 1. Nanodiscs are even without having cholesterol. Thus the particles turned out to a disc shaped particles of uniform size and configuration [41].

**Nanodiscs in membrane protein studies**

Membrane Proteins have posed a greater challenge in different functional as well as biochemical studies. The major challenge lies in the difficulty to study membrane proteins from a mechanistic viewpoint. This is because most of the chemical as well as biophysical methods which are relevant and appropriate to soluble enzymes may fail while coming in contact with the insoluble masses [42]. If the membrane protein is found in a solubilized condition, then it makes the processes like purification, assay, different biophysical and biochemical techniques, structural manipulations, spectroscopical procedures etc. simpler and also helps in sustaining the goal protein in a very stable condition [43]. Over the past years, detergents were the main agents used in solubilisation of membrane proteins and thus ultimately resulted in the formation of micelles containing detergent, membrane proteins and lipids. But the use of detergents has raised a major threat to the constancy of these membrane proteins [44]. Also the micellar stage of detergents can restrict the assay procedures and also exhibit unwanted optical properties such as in absorbance, scattering of light etc... There is also procedural difficulties with the usage of detergents and may result in denaturation as well as inactivation of the proteins (membrane proteins co concentrating with the target). Basically we can say that the traditional use of detergents in membrane protein studies posed some major challenges [45]. Mainly it interferes with the stability of the membrane protein of interest. As said above, it can not only interfere but also impede the different assay procedures. Particular phospholipids are being regulated and required in the functioning of these membrane proteins. The limitations of these detergents resulted in the usage of liposomes for the purpose to incorporate the membrane proteins. But the liposomes also had their own limitations. The liposomes was presented a large surface area, sometimes unstable and also hard to prepare with an exactly controlled stoichiometry and size.

The limitation with liposomes paved the way for the arousal of a new system termed nanodiscs [46]. In this system, the target...
Membrane protein is transitorily made soluble in a detergent. The process is carried out in the presence of phospholipids and a helical protein belt, MSP which is amphipathic in nature and the belt surrounding the phospholipid bilayer. Then polystyrene beads which are hydrophobic in nature are added to the above system. This results in the elimination of detergents in such a way that the detergents get adsorbed on the surface of the beads. Thus the membrane protein is being placed in a native condition and the whole entity is made soluble with the help of the surrounding MSP belt. The major advantage of nanodisc technology is that it can be synthesised in different size and stoichiometry depending on the size of MSP belt used [47]. The nanodisc technology can also be made applicable to different classes of membrane Proteins. The major member among them is the MSP. What is being basically done is that monomerisation of the membrane proteins, and then we will conduct the solubilisation process. Finally, we will do the incorporation process. The protein is being incorporated into membrane environment [48]. By engineering different classes of Scaffold Proteins, different ranges of nanodiscs such as smaller, larger and medium sized nanodiscs can be constructed [49]. This can be achieved by the exact measuring of the give and take of the proteins and lipids within the nanodiscs, by the structural analysis of the consistency and uniformity of the lipoprotein complexes within them and also by explaining the kinetics of the lipo protein assembly by the use of experimental as well as theoretical procedures. The major as well as the most striking advantage of using nanodiscs is that it can be made in different size and define stoichiometry using different MSP [50]. The one dimensional view of a nanodisc has been shown in fig. 1 [8].

Advantages of nanodiscs over other systems

Nanodiscs have many advantages when compared to other systems. The major ones being the provision of a native environment, stability, helps in the solubilisation of membrane proteins etc. Nanodisc system provide contact to both surface of membrane and also helps in the oligomerisation of membrane proteins. The major advantages are as shown in table 1 [24-27]

Table 1: Advantages of nanodiscs over other systems

| Advantage                                                                 | Nanodiscs                                                                 | Other systems                                                                 |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Offers very stable environmental conditions for the membrane proteins    |                                                                          |                                                                              |
| Enhances homogeneity of particles over microsomes                         |                                                                          |                                                                              |
| The membrane proteins remain uniformly dispersed in aqueous solutions    |                                                                          |                                                                              |
| Nanodiscs can be synthesised in different size and also in different stoichiometry. It is dependent on the length of the MSP belt that is being used. |                                                                              |
| Proteins on the membrane surface can be better oligomerised using nanodiscs |                                                                          |                                                                              |
| Nanodiscs show better stability than the conventional methods and also over liposomes. |                                                                          |                                                                              |
| Nanodiscs provide contact to both margins of the membrane.                |                                                                          |                                                                              |

Materials required for nanodisc synthesis

Materials required include MSPs, phospholipids and polystyrene bio beads [51-54].

Membrane scaffold protein

The membrane scaffold protein is the improved Apllo A1 which is made using recombinant technology from human blood, is expressed purified [48, 29]. The choice of MSP depends on the size of nanodisc required.

Phospholipids

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine are some of the most commonly used phospholipids in nanodisc synthesis. Generally zwitterionic and negatively charged lipids are mixed to enhance mimicking the cell membrane. Bacterial membrane lipids have also been used for incorporation of bacterial proteins into nanodiscs [30].

Polystyrene beads

Beads must be washed with water and then using ethanol.

Synthesis of nanodisc

The first step involved in the synthesis of nanodisc is to collaborate together the MSP of appropriate class with the particular phospholipid or the detergent micelle. It is then followed by the elimination or removal of the detergent used [56]. As mentioned earlier, the size and stoichiometry of the nanodisc is being determined by the length of the scaffold protein that has been used. The MSP is actually a belt like structure that is wrapped around the protein. Next step is the determination of the phospholipid: MSP. This is a crucial step for the efficacious assembling of nanodiscs. This has to be determined exactly for each new combination of MSP, the phospholipid and the protein. Then the protein solubilised in detergent is added to the disc mix. It has to be made ensured that this step is being done prior to the removal of the detergent. Thus the membrane protein has been merged into the nanodiscs. The major steps involving solubilisation, detergent removal as well as assembling helps in the effective nanodisc synthesis and has been shown in fig. 2.

Reorganisation of proteins into nanodiscs in three different ways

Cell free reaction system+arranged nanodiscs

Detergents are not needed in this method of protein reconstitution. Membrane proteins can be made in cell free expression system. Preassembled nanodiscs are combined with the cell free reaction system and it ultimately results in joining or incorporation of the membrane protein into nano disk [57].
Previously solubilised membrane protein + detergent + MSP protein + phospholipid

MSPs and phospholipids are added subsequently to the purified proteins. Nanodiscs incorporated with membrane proteins are formed and can be purified by chromatographic techniques.

Detergent + MSP + protein expressed membrane

To the protein expressed membrane, MSPs and detergents are added. Phospholipids, proteins etcetera in the membrane and MSPs align together to form the nano disc complex. This is beneficial in proteomic studies. Single nanodisc–protein complexes can also be isolated by various chromatographic techniques (affinity chromatography) [58, 53].

Nanodisc reconstitution mixtures

Nanodisc reconstitution mixtures can be prepared by using an efficient way involving chloroform, lipid stocks, nitrogen steam, and solvent removal by vacuum dessicator, lipid drying, addition of sodium cholate buffer and finally vortexing and sonicating as seen in fig. 3 [53, 22, 59-61].

Applications of nanodiscs

The modern innovations in nanodiscs and cancer therapy

The evolution of an eminent therapy for cancer has been one of the primarily focussed aspects not only in the medical field but also in terms of the society. Despite the availability of many cancer vaccines in the medicine field including peptide based vaccines for cancer therapy, their utility in humans was found to be limited only. The latest innovations in chemotherapy include personalised immune therapy which has been achieved with the aid of very specific neo antigens. The milestone behind this recent innovation was found to be due to the exome sequencing of tumour cells. The major limitation of this methodology was that the procedure of strongly activating the CD 8 alpha+responses (in the Cytotoxic T lymphocyte Cells) was absent. The innovatory finding signalled first of all to the preparation of nanodiscs which has the property of mimicking the HDL [62]. Then the next step was to incorporate and pair the antigenic peptides along with the suitable adjuvants into the nanodiscs. The main purpose of this step is to enhance the recruitment of antigen and adjuvant to the lymphoid organs. It also results in sustaining the antigenic concentration within the cells [63]. Nanodiscs can be occasioned up to 47 doubling greater rates of neo antigen specific Cytotoxic T lymphocyte Cells (CTL s) than solvable vaccines. The subsequent nanodiscs can also be constructed with a rate more than 31 fold frequency of the adjuvant which is the stronger one. This procedure has been utilised in the trials that has been conducted clinically. It has been found that the growth of the tumour cells can be potentially eliminated by efficiently utilising this method [64]. The method consists of vaccines involving multi epitopes. The link between the evolution of multi epitope vaccines and the inhibition of tumour growth is the generation of broad spectrum responses of T lymphocyte cells. The use of nanodiscs resulted in the elimination of MC 38 and B16 F10 tumours. This innovatory process resulted due to the pairing of subsequent nanodiscs with anti PD1 and anti CTLA 4 therapy for cancer [65, 73-74]. Thus these revolutionary findings that correlate nanodiscs into the most useful accessory for cancer therapy paved the way of the evolution of this upcoming system into a powerful strategy for cancer personalised immunotherapy. The tumour cells get mutated to form neoantigens. The most basic idea behind a cancer vaccine involving nanodisc is as follows: The nanodisc is being encumbered with these mutated neoantigens [66]. This neoantigen loaded nanodisc signal the destruction of the cancer cells. Unlike the traditional preventive vaccine, this new vaccine concerning nanodisc is a therapeutic vaccine. The principle behind this vaccine is that it will bout against the cancer cells in a personalised method by the prompting of one’s immune system [67].

Nanodisc technology represents a revolution in the capability of our understanding of the functionality of membrane proteins and provide advanced tenacity of strategic molecular events basic to a mechanistic understanding of the crucial metabolic as well as signalling functions and further there will be implication for the upcoming of much more effective intervention when those basic events get disrupted during some particular pathological conditions [68].

Other applications

Other major applications of nanodiscs include Nuclear magnetic Studies [69-70], surface plasma Resonance Studies [71-74], protein activation studies [53], binding studies of proteins etc.
CONCLUSION
There has been constant experimenting on drug delivery studies ranging from nanoparticle drug delivery to microbial infectious sites to macromolecule studies of tissue regeneration. In the current scenario that finds drug targeting as one of the major challenges, nano drug delivery has a very important role especially in crossing the Blood Brain Barrier. The evolution of nanodiscs in membrane protein studies thus serve as a promising tool in novel drug delivery. The provision of a more native and genuine environment of nanodiscs when compared to others like liposomes, detergent micelles etc. once again proves the promising aspect of nanodisc. Nanodiscs can be synthesised in different size and also in different stoichiometry. It is dependent on the length of the MSP belt that is being used. Thus overall it can be stated as a promising novel aspect, in this era where a number of different tools including hydrotropes are under studies.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
Declared none.

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