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Review Article

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Microbubble: As a Theraputic and Dignostic Tool

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Abstract

Gas filled microbubbles are well known as ultrasound contrast agents for medical ultrasound imaging and for non-invasive delivery of drugs and genes to different tissues. Microbubbles designate air or gas filled microspheres suspended in a liquid carrier phase which generally results from the introduction of air or gas. The liquid phase contains surfactants to control the surface properties as well as stability of the bubble. Microbubbles are manufactured from biocompatible materials, so they can be injected intravenously. Micro bubbles have an average size (1-8 µm) less than that of RBC's i.e. they are capable of penetrating even into the smallest blood capillaries & releasing drugs or genes, incorporated on their surface, under the action of ultrasound. Ultrasound radiation are used which are non hazardous. Most of the physicians today prefer imaging with ultrasound in combination with microbubbles compared to other diagnostic techniques for low cost and rapidity. The ultrasonic field can be focused at the target tissues and organs; thus, selectivity of the treatment can be improved, reducing undesirable side effects. Recently, targeting ligands are attached to the surface of the microbubbles, which have been widely used in cardiovascular system, tumour diagnosis and therapy. This review focuses on the characteristics of microbubbles that give them therapeutic properties and some important aspects of ultrasound parameters that are known to influence microbubble-mediated drug delivery. In addition, current studies involve discussion of novel therapeutical application of microbubbles.

Key words: Microbubbles, Ultrasound, Contrast agent, Targeted drug delivery

Introduction

The main goal of drug delivery and targeting by microbubble is to improve the efficiency of drug action in the region, where the disease cells are arised and reducing undesired adverse effects, such as toxicity, in the healthy tissues. For the drug action and/or deposition in the targeted region various external energy field applied, like light (photodynamic therapy), neutron beam (boron neutron capture therapy), magnetic field (targeted accumulation of magnetic drug carrier in the tissues close to the magnet) or mechanical energy. In order to improve drug action we applied mechanical energy in form of ultrasound irradiation. Ultrasound improves drug delivery into tissue and cells [1]. Microbubbles are known as contrast agents for ultrasound (US) diagnostics and imaging presence of ultrasound energy deposition foci in the tissues. They increase the cell permeability by perturbing cell membranes. Recently, Microbubbles are anticipated to find further uses in therapy as efficient and safe targeted deliverers of drugs and genes. Microbubbles are gas-filled colloidal particles, with a size range of 100 μm [2]. The intrinsic compressibility of microbubbles is approximately 17,000 times more than water. They also scatter ultrasound very strongly, so they are known as ultrasound contrast agent [3].

Gas-filled microbubbles administered by intravascular route can serve as cavitation nuclei. So they have wide range of ultrasound- mediated drug delivery applications [4]. The main application of ultrasound and microbubbles is to targeted drug and gene delivery in specific region of disease. Under exposure of sufficiently high-amplitude ultrasound, these targeted microbubbles would carry a drug or gene to a specific area of interest and then ultrasound is used to burst the microbubbles, causing site-specific delivery of the bioactive material [5,6]. Their structure (Figure 1 & 2) comprises of [5, 6, 7, 8]

1) Innermost Gas Phase
2) Shell Material Enclosing the Gas Phase
3) Outermost Liquid or Aqueous Phase.

1. Gas Phase

A gas core which is wrapped in a more or less flexible shell of protein (albumin), lipid, surfactant or biocompatible polymer (2 to 500 nm thickness). Encapsulated gas microbubbles are well known as ultrasound contrast agents for medical ultrasound imaging. Encapsulation is mainly done for the improve stability against gas loss, dissolution and microbubble coalescence. A surfactant layer increases the half-life of the microbubble by decreasing surface tension, in some to near-zero values. Generally single gas or combination of gases are used in gas phases. Combination gases are used for the stabilization of the microbubbles by causing difference in partial pressure which generates gas osmotic pressure.

In a combination of gases two types of gases are involved:

1. One is the Primary Modifier Gas also known as first gas. Eg. Air, Nitrogen
2. Gas Osmotic Agent also known as second gas; which is a less permeable through the microbubble surface than the modifier gas.

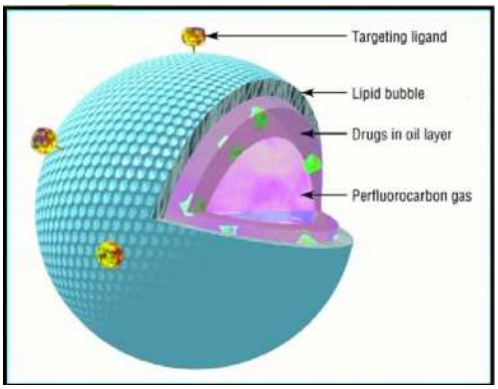

Figure: 1 Components of microbubbles

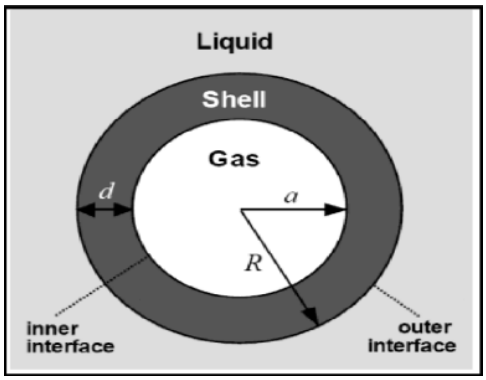

Figure: 2 Various Layers of micro bubble structure

Gas osmotic agent is normally a gas at room temperature or liquid so long as it has a sufficient partial vapour pressure at the temperature of use to provide the desired osmotic effect. Eg. Heavy gases like per fluorocarbons or

sulfur hexafluoride. The gas core is the most important part of the ultrasound contrast microbubble because it determines the echogenicity. When gas bubbles are caught in an ultrasonic frequency field, they compress, oscillate and reflect a characteristic echo- this generates the strong and unique sonogram in contrast-enhanced ultrasound. Heavy gases are less water-soluble so they are less likely to leak out from the microbubble to impair echogenicity. Therefore microbubbles with heavy gas cores are likely to last longer in circulation. The size is smaller than red blood cells, which allows them to flow or penetrate through the small blood capillaries and releasing drug and genes under the action of ultrasound field.

2. Shell Material

The stiffness of the microbubbles depends on the composition of the shell and their resistance to rupture in the ultrasound pressure field. The shell material encapsulates the gas phase. Its major role in the mechanical properties of microbubble as well as diffusion of the gas out of the microbubble. The elasticity or compressibility of microbubbles also depends on shell material. Selection of shell material determines how easily the microbubble is taken up by the immune system. More elastic the shell material requires more acoustic energy to withstand before bursting or breaking up, this increases the residence time of these bubbles in body. More hydrophilic the shell material, more easily it is taken up by the body this decreases the residence time of these bubbles in the body. This reduces the time available for contrast imaging.

Eg: proteins like albumin, carbohydrates like galactose, phosholipids like phosphotidylcholine, etc.

3. Aqueous or Liquid Phase

The external, continuous liquid phase contains a surfactant or foaming agent. Surfactants suitable for use include any compound or composition that aids in the formation and maintenance of the microbubble membrane by forming a layer at the inter phase. The foaming agent or surfactant may comprise a single component or any combination of compounds, such as in the case of co-surfactants.

Eg: Block copolymers of polyoxypropylene, polyoxyethylene, sugar esters, fatty alcohols, aliphatic amine oxides, hyaluronic acid esters & their salts, dodecylpoly (ethyleneoxy) ethanol, etc. **Nonionic Surfactants**: Polyoxyehylene polyoxypropylene copolymers Eg. Pluronic F- 68, polyoxyethylene stearates, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerol polyethylene glycol ricinoleate etc. **Anionic Surfactants**: Fatty acids having 12 -24 carbon atoms Eg. Sodium Oleate.

4. Other Components

The various other components that may be incorporated in the formulation include osmotic agents, stabilizers, chelators, buffers, viscosity modulators, air solubility modifiers, salts and sugars can be added to fine tune the microbubble suspensions for maximum shelf life and contrast enhancement.

The microbubbles which mostly contain oxygen or air can remain suspended in water for extended period. The first generation microbubbles (e.g. Albunex®) were filled with air. Because of the high solubility of air in blood and a thin (10–15 nm) protein shell coat that was not a good barrier against gas diffusion, these microbubbles disappeared from the bloodstream within seconds after administration. Inert, high molecular weight gases like per fluorocarbons or sulfur hexafluoride are used in second and third generation contrast agents. Their decreased solubility and low diffusion coefficient prolong the lifespan of microbubbles within the circulation.

Characterisation of Micro-Bubble [9]

1. Microbubble Diameter & Size Distribution: Measure by Laser light Scattering, Scanning Electron Microscopy and Transmission Electron Microscopy.

2. Shell Thickness: By the coating the microbubbles with the fluorescent dye using Fluorescent Microscopy against a dark background.

3. Microbubble Concentration: Measure by counting the number of microbubbles per ml by using the Coulter Counter Machine.

Air Content by densitometry: The content of air encapsulated within the microbubbles in the suspension samples is measured by oscillation U-tube densitometry with a DMA-58.

Methods to Prepare Microbubbles: [9, 10, 11,12]

The various methods like Cross Linking Polymerization, Emulsion Solvent Evaporation, Atomization & Reconstitution, Sonication that can be used for the preparation of these microbubbles.

1. Cross Linking Polymerization

In this a polymeric solution(2% aqueous solution of PVA) is vigorously stirred, which results in the formation of a fine foam of the polymer which acts as a colloidal stabilizer as well as a bubble coating agent. The polymer is then cross linked, after cross linking microbubbles float on the surface of the mixture. Floating microbubbles are

separated & extensively dialyzed. (By adding HCl or H2SO4 as a catalyst, the cross linking reaction is stopped by neutralization of the mixture and microbubbles are then separated.)

2. Emulsion Solvent Evaporation

In this method two solutions are prepared; one is an aqueous solution containing an appropriate surfactant material which may be amphilic biopolymer such as gelatine, collagen, albumin or globulins. This becomes the outer continuous phase of the emulsion system. The second is made from the dissolution of a wall forming polymer in a mixture of two water immiscible organic liquids. One of the organic liquids is a relatively volatile solvent for the polymer & the other is relatively non-volatile nonsolvent for the polymer. The polymer solution is added to the aqueous solution with agitation to form an emulsion. The emulsification step is carried out until the inner phase droplets are in the desired size. It is the droplet size that will determine the size of the microbubble.

3. Atomization & Reconstitution

A spray dried surfactant solution is formulated by atomizing a surfactant solution into a heated gas this result in formation of porous spheres of the surfactant solution with the primary modifier gas enclosed in it. These porous spheres are then packaged into a vial; the headspace of the vial is then filled with the second gas or gas osmotic agent. The vial is then sealed, at the time of use it is reconstituted with a sterile saline solution. Upon reconstitution the primary modifier gas diffuses out & the secondary gas diffuses in, resulting in size reduction. The microbubbles so formed remain suspended in the saline solution & are then administered to the patient.

4. Sonication

Sonication is preferred for formation of microbubbles, i.e. through an ultrasound transmitting septum or by penetrating a septum with an ultrasound probe including an ultrasonically vibrating hypodermic needle. Sonication can be accomplished in a number of ways, Eg. A vial containing a surfactant solution & gas in headspace of the vial can be sonicated through a thin membrane. Sonication can be done by contacting or even depressing the membrane with an ultrasonic probe or with a focused ultrasound "beam". Once Sonication is accomplished, the microbubble solution can be withdrawn from the vial & delivered to the patient. Sonication can also be done within a syringe with a low power ultrasonically vibrated aspirating assembly on the syringe.

How Microbubbles Work? [3, 13]

They resonates the ultrasound beam. So pressure of sound wave changes and due to this they rapidly contract and expand. We used high frequencies for diagnostic purpose. These high frequencies make them several thousand more reflective than normal body tissues **(**Figure 3). The resonance that microbubbles produce has several special properties that can be exploited to improve diagnosis. Ultrasound scanners can be tuned to "listen" to these harmonics, producing strong preferential imaging of the microbubbles in an image.

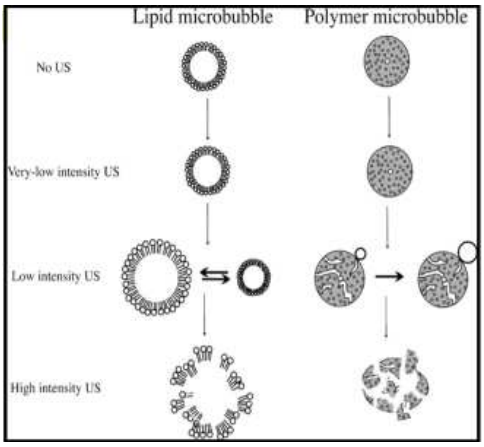

Figure: 3 Schematic representation of lipid (left column) and polymer (right column) microbubble interaction with ultrasound of increasing intensity (top to bottom).

Applications:

1. Imaging Application [3, 14]

Ultrasound is extremely sensitive to the presence of microbubbles. In fact, cavitation imaging and other techniques are capable of detecting a single microbubble. Ultrasonography is currently the most widely used diagnostic medical imaging modality. It is non-invasive and relatively low in cost and it uses portable, real-time imaging equipment. It also avoids hazardous ionizing radiation. An ultrasound transducer placed on the skin or inside the body broadcasts ultrasound pressure wave pulses, which are partially reflected or scattered by the interfaces between different tissues or structures in the body. Some of the scattered sound waves return to the transducer. The imaging system converts these signals into electrical pulses and digitizes them. The time intervals between pulse transmission and reception, as well as the speed of sound in the tissue are known. Therefore, an image that is based on scattered sound signals can be generated. However, blood a liquid phase material with low compressibility, scatters ultrasound poorly. Ultrasound images of blood can be improved by the use of contrast agents, which increase the scattering and reflection of ultrasonic waves.

2. Diagnostic Aids [14, 15, 16, 17]

Microbubbles are elastic and compressible, these undergo compression and rarefaction so they can produce acoustic pressure difference between biological tissues and fluids and so they are efficient reflectors of ultrasound, hence used as contrast agents.

These are used as diagnostic aids for:

a) Organ Edge Delineation b) Inflammation c) Cancer d) Liver

e) To scan the tumors arising in the body f) Used for imaging the gall bladder stone.

Advantages:

1. Inexpensive as compared to other diagnostic agents.
2. No use of ionizing radiation.
3. Faster and accurate as compared to other diagnostic techniques.

3. Targeted Drug Delivery [18, 19, 20, 21]

When we apply ultrasound, microbubble oscillates, so cavitation occurs resulting in breakup of microbubble. By this way drug is released which is incorporated in microbubble.

Two factors which are taken into account for drug delivery are:

1. Incorporation of drug into these microbubbles
2. Drug release from these microbubbles

1. Incorporation of Drug into Microbubbles

By various ways drug molecules (Figure 4) is incorporated in microbubble like,

A. Drug molecules can be incorporated within the bubble.

B. Drug molecules can be incorporated within the bubble membrane or shell material of the microbubble.

C. Drugs can also be attached to the shell of the microbubble (Eg. by noncovalent bonds)

D. These can also be attached to the microbubble surface via a ligand. (Eg. avidin-biotin complex).

E. Also if the microbubble is made up of multiple layers it can also be incorporated within the various layers of these microbubbles.

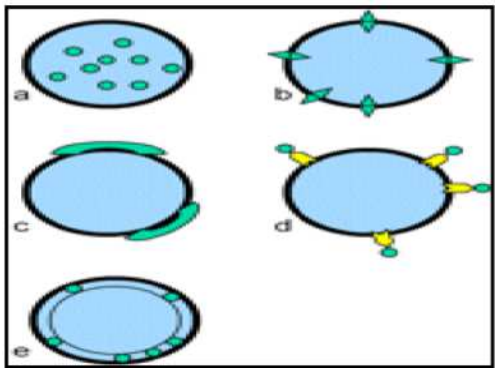

Figure: 4 Incorporation of drug

2. Drug Release from Microbubbles

Microbubbles are also used as a carrier in drug delivery. When we apply ultrasound microbubble oscillates, so cavitation occur which results in bursting or breakup of the microbubble on application of ultrasound. On cavitation

the body fluids start insonating creating acoustic cavitation. Further by the oscillation in ultrasound they give rise to small eddies, these eddies give rise to micro streaming or micro jets. Due to this, microbubble increases the permeability of the cell membrane & drug transfer occurs across the membrane. Sometimes the microbubbles may also be phagocytised by the cell membrane resulting in drug release.Another mechanism is fusion of the phospholipids microbubble with the phospholipids bilayer of cell membrane resulting in delivery of the drug or genes directly into the cytoplasm of the cell membrane.
The figure 5 & 6 shows drug delivery via the microbubbles

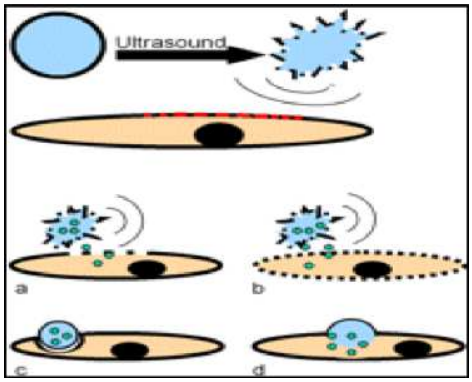

Figure:5 Drug deliveries via Microbubbles

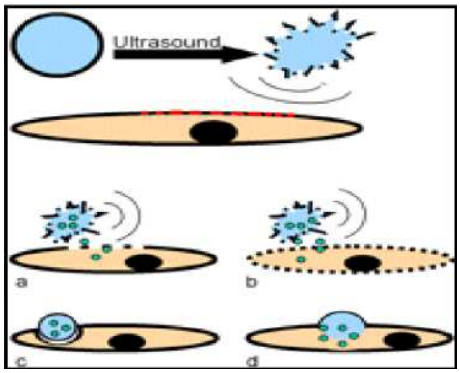

Fig:6 Targeted microbubble,Connected by ligand & receptor

A. Drug delivery by cavitation
B. Drug release by cavitation as well as increasing the permeability of cell membrane
C. Phagocytosis of the microbubble by cell membrane
D. Fusion of microbubble with the cell membrane.

Advantages

1. Smaller dose is required compare to conventional.
2. A decrease in the side effects as the drug is released near its target & due to the small dose especially for antineoplastic drugs.
3. By attaching various ligands these can be used for targeted drug delivery.

4. Gene Delivery [22, 23]

The another main application of microbubble in gene delivery, because
1. Microbubbles are metabolically inert.
2. When injected into the body, they do not produce any immune response.
3. Also the gene encapsulated or attached to the microbubble is not digested by various enzymes.

Charged drugs can be stabilized in or onto the surfaces of microbubbles by electrostatic interactions lipid-coated microbubbles to bind DNA. In DNA the sugar phosphate group is present, is a polyanion (i.e. negatively charged). So DNA is bound to cationic (positively charged) microbubbles. The gene is released when ultrasound energy cavitates the microbubble (Figure 7).

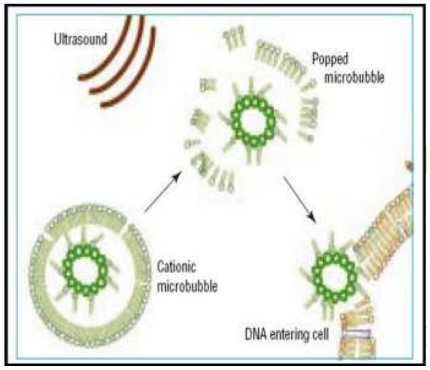

Figure: 7 Gene Delivery

Ultrasound can be focused upon almost any organ and hence this approach is used to deliver targeted gene therapy in cardiovascular conditions such as such angioplasty restenosis and in many other clinical situations. The presence of gas in the gene-filled microbubble allows ultrasound energy to "pop" the bubble. An energetic wave is then created which allows the genetic material to enter surrounding cells. Microbubbles are currently used clinically as contrast agents in ultrasound diagnostics and experimentally as drug or nucleic acid carriers. Localized delivery, drug release and tissue penetration can be achieved by local application of ultrasound leading to microbubble disruption. In cell culture, magnetic microbubbles are sedimented on target cells by magnetic force and bubble disruption and gene delivery is triggered by the application of ultrasound of 1 MHz Magnetic microbubbles loaded with appropriate agents can considerably improve drug deposition and the site specificity of delivery. In particular, magnetic microbubbles may offer a combination of therapeutic intervention with molecular imaging.

5. Intravascular Applications

A. Angiogenesis

Certain integrins such as AlphaV BetaIII are expressed in angiogenesis. Microbubbles targeted to AlphaV BetaIII can be used to measure the temporal expression of this integrin in association with angiogenesis. A contrast agent targeted to endothelial- based markers of angiogenesis might be used to improve diagnosis and treatment of disorders affected by angiogenesis [3].

B. Vulnerable Plaque

Vulnerable plaques are those that have been infiltrated by macrophages, and are undergoing inflammation. Inflammation can lead to rupture of vulnerable plaque and formation of thrombus, as in stroke and myocardial infarct. Vulnerable plaques may lie hidden as unseen threats, liable to cause morbidity and sudden death. Vulnerable plaque has been successfully detected using targeted microbubbles in combination with ultrasound [3,23].

6. Extravascular Applications

For targeting beyond the vasculature, the particles usually need to have very small diameters. This requirement can be met by nanobubbles and per fluorocarbon emulsions. Targeting ligands can be incorporated into these systems. The ligands may then be directed to targets on the surface of cells or in the intercellular matrix outside the vasculature. Very small bubbles (i.e. below 500 nm diameter) can be detected with high frequency imaging and other ultrasound techniques. When sufficient quantities of these structures are delivered to a tissue, they can act as specular reflectors and greatly increase the backscattered signal. Smaller quantities can be detected with cavitation imaging. Nanobubbles may make it possible to target, for example, epithelial cells in carcinoma. Such agents might be used not only to detect defect in body but also to treat diseases such as cancer [3].

A. Sonothrombolysis

Microbubbles accelerate the rate of Sonolysis. Enhancement is greater for the targeted molecular ultrasound contrast agent than for the non-targeted agent. Microbubble enhanced SonoLysis may have potential clinical applications for rapid and safe treatment of vascular thrombosis. This could have clinical applications for treating myocardial infarction, stroke and deep venous thrombosis. Sonolysis using ultrasound at 200 kilohertz with microbubbles restored blood flow [24].

B. Passing the Blood Brain Barrier

Ultrasound activation might be used to afford precise entry into the CNS by controlling the BBB at the molecular level [3,24].

C. Increase the Efficiency of Cancer Treatment

Laser-induced bubble formation around nanoparticles may play a crucial role in selective laser nano photothermolysis of cancer cells targeted with nanoparticles [3].

D. Prostate Cancer Detection

We assessed contrast enhanced colour Doppler Ultrasonography by means of a microbubble ultrasound contrast agent to detect tumour vascularity and improve the diagnosis of prostate cancer. The use of a microbubble ultrasound contrast agent for transrectal colour Doppler targeted biopsy significantly improved the detection of prostate cancer compared with systematic biopsy following conventional grey- scale Ultrasonography [3,25].

E. Leukaemia Treatment

Acute myeloid leukaemia (AML) is a quick progressive cancer. Normally high doses of cytotoxic drugs is given in the treatment and in selected cases hematopoietic stem cell transplantation. These high intensive therapy regimen involve toxicity problems for a lot of patients over 60 years.Since the treatment can be so exhausting, administration of direct drug delivery may allow the patient to receive disease controlling treatment with palliative intentions. Direct drug delivery also gives the opportunity to use stronger drugs than otherwise would be too toxic for the patient [26, 27, 28].

Conclusion

In this article, we described the most important issues in the development of contrast agents. Firstly, specific acoustic and biological properties make microbubbles a promising tool as a vehicle for drug and gene delivery. The targeted microbubbles have a great use in future. Microbubbles have been targeted to receptors of leukocytes and blood clots, and may be used in diagnostic imaging of thrombo-embolic or inflammatory processes. These microbubbles can be used as a vehicle for drugs or genes and local delivery can be achieved by destruction of microbubbles with ultrasound. Intravascular microbubbles can improve drug penetration into tissues when combined with focused ultrasound treatment, such as through the blood-brain barrier. Drug substances, including plasmid DNA, can be attached to or incorporated in the microbubble particles for ultrasound-triggered release in the insonated organs and tissues. Overall, ultrasound-assisted drug delivery combined with microbubble contrast agents will aid in the treatment of debilitating diseases.

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