

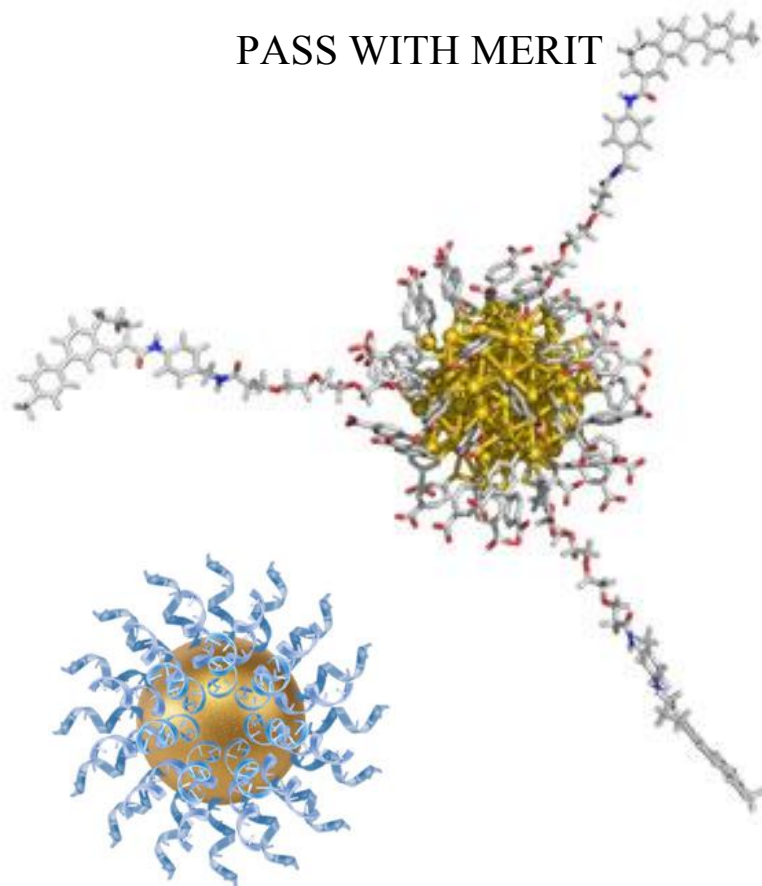
NANOTECHNOLOGY – THE PROMISING FUTURE FOR
MEDICINE:

AN INVESTIGATION INTO THE POSSIBILITIES OF THE USE
OF PLASMONIC NANOBUBBLES IN THE THERANOSTICS
AND TREATMENT OF PROSTATE CANCER

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Abstract

The study of nanotechnology has already begun to open up a wide horizon for the future of medicine. The use of artificial devices and complex systems at the molecular level has led scientists all over the planet to understand the possibilities for diagnosing, treating and preventing many serious diseases and medical conditions, for example in the detection and targeting of cancer cells in the prostate. Although the presented methods have inferred the perfect cure for this type of cancer, many of them consist of inefficient prevention of spread and have led to side effects such as erectile dysfunction and urinary infections. Worldwide around 258,000 men died from prostate cancer in 2008, with many cases due to late diagnosis [1]. But Rice University in Texas have tested a technique involving Plasmonic Nanobubbles which combine successful therapeutics and diagnostics into a field known as “theranostics” which has proven to be able to detect the cancer and destroy it effectively without the risks mentioned above. This report aims to discuss the benefits and details of this new theoretical method and to acknowledge the ethical issues or risks associated in parallel.

Introduction

The development in research for nanotechnology has expanded exponentially over the last 20 years. Many scientists are predicting an approaching “golden age” for medicine, whereby we can use nanotechnology to solve a whole spectrum of cases across the board of medicine. Richard Smalley, founder of the Centre for Nanoscale Science at Rice University quoted “Over the past century we have learned about the workings of biological nanomachines to an incredible level of detail, and the benefits of this knowledge are beginning to be felt in medicine” [2]

Nanotechnology works at the molecular level, where we are able to construct complex devices the size of a few atoms, for many purposes. From nanorobots to drug delivery systems (DDS), the small scale and specificity of the particles are able to delve into the very fine detail of molecular biology. By controlling such objects we are able to use nanotechnology to our advantage in all aspects of medicine. Hence, nanomedicine is an ever-expanding field of study that is surely becoming a central sector of the medical world, in the areas of diagnosis, treatment, surgery, clinical work and research into biological coding. The potential for nanomedicine is enormous.

In short, a definition of nanomedicine can be:

“The design and manipulation of nanoparticles (i.e., nanotechnology), particularly as applied to the medical diagnosis and treatment of disease. Carbon-based nanoparticles such as nanotubes or buckyballs (see fullerene) are considered nanomedicine candidates.” [3]

Nanomedicine is already promising to be a huge help to diagnosing diseases. Man-made devices at the nano-scale are carefully made to be stable, compatible and controllable to identify a particular virus, antigen or affected cell in the body. Nanoparticles have to target particular sites in the body to test for diseases, especially types of cancer. Nanoparticles can be used in a technique called molecular imprinting, whereby the devices interact with certain receptors on the membranes of cells that respond to a particular disease, including enzymes. Once the particle has an imprint, it can be injected into the bloodstream and will bind to the particular virus that usually binds to that receptor with the complementary shape. Research is also covering Quantum Dots, which are particles the same size as DNA, which show fluorescent lights at different sizes. When they bind to specific biological molecules being tested for, they can be a thousand times brighter than regular dyes, which makes diagnosis clearer and more precise in screening. Not only will this improve diagnosis and recognition of a particular infection, but it will make targeting the disease much easier.

Nanotechnology is also making a big influence on surgical procedures. Nanorobots and particles are able to observe and get into the smaller compartments of major organs where surgical instruments are too large to permit. Nanorobots have molecular sized computer systems and on-board sensors so that the surgeon can control the devices to repair or cut through a particular tissue, or even unclot vessels in the blood or lymph. Surgery would hence become less invasive and as a result we would be able to give more developed treatment in

certain diseases, where open surgery would lead to infections or serious risks for example in cardiac operations as opposed to nanosurgery.

In relation to this aspect, nanotechnology has huge potential for the repairing of tissues or organs. Nanoparticles carrying specific biological materials can work together to rebuild and link tissues damaged for many reasons. Given that the procedure could be perfected, this would not only increase the rate in which patients are cured, but it would give more confidence to future patients given a higher survival rate where invasive surgery is too risky.

Tests have also proved nanoparticles to be very effective drug delivery systems (DDS) which improves drug bioavailability. Nanoparticles would be able to be injected into the patients' bloodstream and would target specific cells and then specific parts of those cells to deliver the drug. This reduces the problems that arise normally, where drugs affect cells not in need of the drug which can mean medication is more of a problem than a cure or prevention. In most circumstances much of the drug doesn't reach the target and is wasted, but nanoparticles let the drug pass through vessel walls and reach the target efficiently without being used up elsewhere.

A popular example of this is in the treatment of cancer cells. Nanoparticles with particular polymers on the surface, usually a type of RNA can target cancer cells and bind to them, and once activated with a particular laser, for example ultrasound, the particle will destroy that cell. This technique is similar to the one I will be discussing later. Usually a patient will have to experience repetitive doses of chemo or radiotherapy which can have many side effects, and which is less efficient at targeting the cells [4].

Current research in nanomedicine has a large part in the functioning of nanotubes. Carbon Nanotubes (CNTs) are allotropes of carbon in a cylindrical structure. They exhibit phenomenal strength, being stronger than steel in many cases, yet a lot less dense. The tubes are members of the fullerene structural family, which links to the spherical "buckyballs" which can be found on the end of nanotubes. These structures have potential to be very effective in drug delivery. The large volume inside the nanotube can be used to encapsulate a drug and the small size enables the tube to penetrate cell membranes or bodily vessels without causing any major damage. Current problems with these are their solubility and their short half-life, but these problems are being addressed in the most recent research. This diagram shows how toxins or drugs can be inserted into a foreign body:

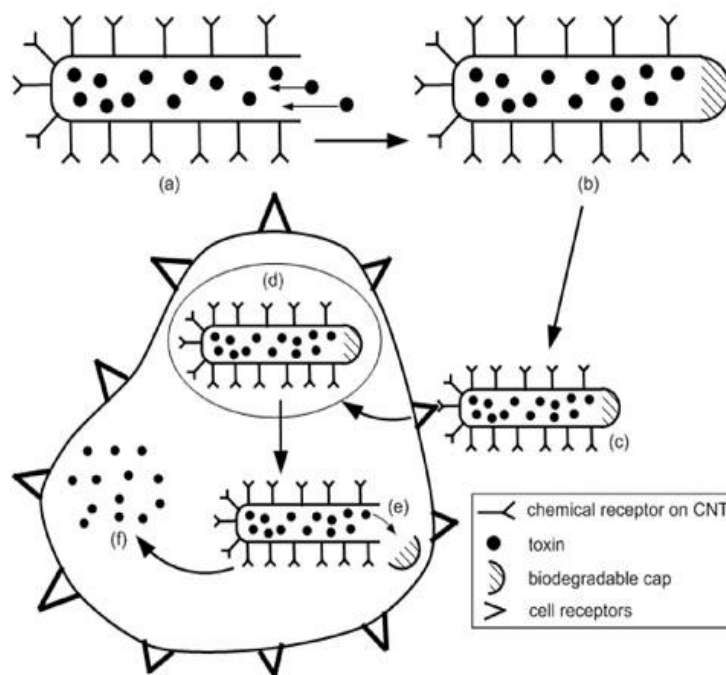


Figure 1. – [6] – the Carbon Nanotube “Magic Bullet”

The diagram outlines the method in which a carbon nanotube can be used to deliver a toxin into a pathogen. The drug is inserted into the volume of the carbon nanotube, which has its own biochemical receptors to target the pathogen. The tube is sealed with a biodegradable cap. Once the antigens on the pathogen bind to the receptor, the nanotube enters the cell via endocytosis and the cap biodegrades inside. The toxins are then released.

This effective way of delivering drugs ensures that only the areas that need the drug are targeted by the nanotube's receptors and if the drug contains very lethal toxins, they don't enter our own cells which might have serious consequences.

Most features of medicine work at the molecular level. From viruses, to DNA, to the capillaries that span the network of our tissues, nanotechnology is able to manipulate many parts of medicine and anatomy for our

benefits. Biology and nanotechnology work at similar sizes and similar levels which makes nanomedicine a topic that will stretch far into the future. [For more reading, I recommend “Advanced Nanomedicine & Nanobiotechnology” by Viroj Wiwanitkit [5]]

Discussion

It is obvious that nanomedicine demonstrates a colossal amount of potential for all aspects of medicine, but scientists are now finding ways in which some of our biggest killing cancers can be managed and prevented efficiently through nanotechnology. Researchers originating from Rice University in Texas have developed an interesting technique to detecting and eliminating prostate cancer cells with use of specific nanoparticles with certain thermal properties that are activated through the use of lasers, which individually target cancer cells and destroy them with fewer side-effects than previous methods. These Plasmonic Nanobubbles are at the very edge of discovery in nanomedicine.

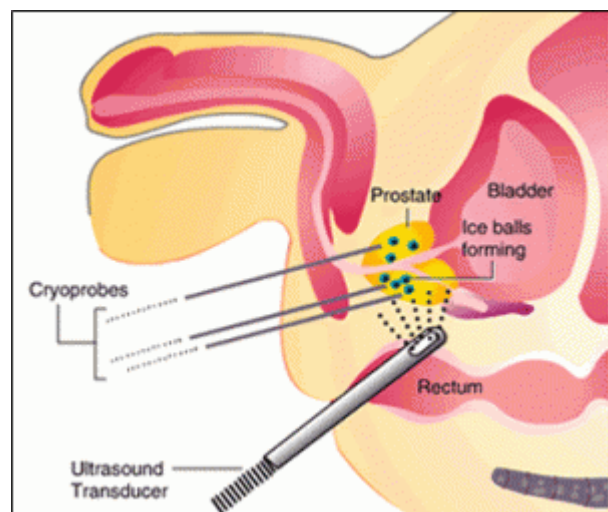
But to understand the theory behind the process, we must appreciate the origins of prostate cancer, its negative impact on our lives and the differences between affected cells and normal cells. The prostate is an area of the male reproductive system that is important in storing seminal fluid. It lies in the pelvis under the urinary bladder, surrounding part of the urethra which carries urine or semen during urination or ejaculation. Due to this location, prostate related diseases often affect urination and ejaculation. Cells in the prostate make up glands to hold and secrete these fluids often mutate in cancer.

The main risk factors for prostate cancer are age and family history. It is more common in men older than 45 years. The average age of diagnosis is at about 70 years [7]. Men with high blood pressure are also likely to develop prostate cancer. It is not just one gene that codes for prostate cancer. Mutations in BRCA1 and BRCA2 have been recognised as primary causes for uncontrolled growth, and these genes also cause breast and ovarian cancer in women. The HPC1 gene, the androgen receptor and the vitamin D receptor are also genes linked to the cause. During the prostatic carcinogenesis, certain cancer-suppressor genes are lost which allows the growth of tumours. The website from reference {7} quoted “70% of men with this type of cancer have lost one copy of the PTEN gene (a tumour suppressor gene)”.

The current treatment for prostate cancer has many limitations and risk factors. It is these side-effects from the treatment which bring about the difficult decision which requires weighing up the benefits of reducing the cancer, or acknowledging the risks of infections, disorders associated as side-effects and possible need for more therapy at a later date. Treatment currently includes active surveillance, (which monitors progress of tumour growth), surgery, (radical prostatectomy), and extreme beam radiation therapy including chemotherapy, cryosurgery or High-Intensity Focused Ultrasound (HIFU). The issue is that these treatments only delay the growth of the cancer and in many cases do not terminate it fully. It often makes males impotent and their quality of life becomes hindered. It can lead to many dangerous side effects such as urinary incontinence and erectile dysfunction which greatly affects the lifestyles of sufferers.

Figure 2. [8]

Figure 2 shows the use of cryotherapy in the treatment of prostate cancer cells. The cryoprobes emit extreme beams of radiation, which, under the action of the ultrasound transducer, freeze the cancer tissues and the procedure then thaws them and freezes them again. This kills the cancer tissue. Yet as mentioned, side-effects can occur and consequently doctors are less confident in using this procedure.



The use of Plasmonic Nanobubbles (PNBs) has proven to be more successful than these therapeutic techniques and has even managed to outcompete the previous method of using nanoparticles in passive detection of cancer cells. The biomedical applications of PNBs use their optical scattering properties for imaging and diagnostics, and use their photothermal properties for therapies through the generation of heat bubbles and acoustic waves. On primary testing, the particles were using passive targeting to root out the cancer cells, yet they could not recognise their specific molecular targets and this resulted in low selectivity and toxic levels of nanoparticles in the body.

The situation was improved by using ACTIVE molecular targeting by the use of antibodies on the particles to couple with receptors on the cancer cells. This method was used in *in vitro* and *in vivo* application of NP based imaging and diagnostics. An antibody-endocytosis based mechanism consisting of gold-based nanoparticles were made. Such clusters were named as Plasmonic Nanobubbles. PNBs are created as transient (short-living) vapour bubbles around intracellular NP clusters, all under the influence of short laser pulses. The laser must be at a very specific threshold to make the thermal properties. The tuneable optical (diagnostic) and mechanical (therapeutic) properties of the devices make them take on a focal role in **theranostics** - "Theranostics is the term used to describe the proposed process of diagnostic therapy for individual patients" [9].

Nanobubbles are created when gold nanoparticles are struck by these laser pulses. The laser overheats the gold particles and evaporates a small volume around the medium to create a small bubble of vapour. The bubbles are bright and can be altered by the power of the laser. The bubbles are therefore visible under a microscope and the diagnosis of cancer cells is effective at picking out individual cells for targeted destruction. In laboratory studies published last year, Lapotko and colleagues at the Laboratory for Laser Cytotechnologies at the A.V. Lykov Heat and Mass Transfer Institute in Minsk, Belarus, applied nanobubbles to arterial plaque. They found that they could blast right through the deposits that block arteries. "The bubbles work like a jackhammer," Lapotko said. [10] The bubbles created expand and collapse in a matter of nanoseconds, but the light emitted is able to be collected to detect cancerous cells.

Once the cancerous cells are identified, a second laser pulse is emitted that causes these bound nanobubbles to explode, or "mechanically ablate". The laser causes the bubble to boil away and confirms the cellular destruction. The bubbles ensure that only the attached cell is destroyed, which differs hugely to radio or chemotherapy, where a lot of living tissue is damaged unnecessarily. The bubbles are converting light energy into mechanical energy.

Tiny gold particles are great at transferring heat and would be a promising tool for creating localized heating in, e.g., a living cell. In new experiments, researchers at the Niels Bohr Institute have measured the temperature of nano-sized gold particles with extreme precision and have examined their ability to melt the lipid membranes surrounding cells, paving the way for dissolving sick cells. The results have been published in the esteemed journal Nano Letters.

Gold nano-particles have a strong interaction with light in relation to their size and it is precisely their physical size that gives them different colours. Its colour is the result of how strongly a gold particle scatters and absorbs light at different wavelengths. Therefore, when the light heats up the gold particle, the colour has significance for its temperature.

The research was conducted in the Optical Tweezers Group at the Niels Bohr Institute. Optical tweezers are sophisticated instruments, which using an extremely focused laser light can trap and hold gold particles on a nanometer scale. A nanometer is a thousandth of a millimetre and therefore very small. The gold particles are between 60 and 200 nanometres in size.

When the particle gets very hot and the bubble collapses, the lipids in the cell membrane (including cholesterol, phospholipid bilayers and other steroids) break down and the cell is killed. This only happens on the

cell that the bubble is attached to. Compared to nanoparticle based testing, these bubbles act at the molecular and cellular level providing much higher localization in the impacts. The diagnosis is therefore more sensitive, the destruction of cells is a more selective decision and in 2 very quick stages we are able to diagnose and treat the cancer together, linking back to the idea of a “theranostic” procedure.

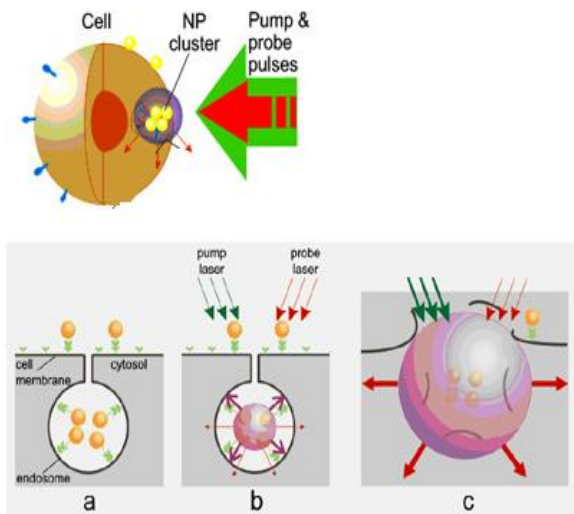


Figure 3 outlines the basics of the procedure. The body of the nanobubble is almost completely engulfed into the cancerous cell, from endocytosis action. The pump laser, (shown as green arrow) is responsible for heating the particle to give off the light energy to be detected. The second laser causes the bubble to collapse and damage the inside of the cell, permanently killing it. The bubble has a nanoparticle cluster at the core which consists of gold particles.

Figure 3A [11]

The manual control of the fluence (electromagnetic radiation energy) of each laser pulse allows us to optimize the excitation of the multi-NP cluster for improved selectivity of PNB generation, whereas excitation with a single pulse does not offer this opportunity.

From the biomedical point of view the above features can be considered as a reduction of the radiation impact on surrounding tissue and improvements in PNB selectivity in a heterogeneous cell environment when different NPs are targeted with the different vectors (e.g. antibodies, peptides). Optical generation and detection of the PNBs was performed with a previously developed photothermal laser microscope equipped with a dual pulsed laser: each pulse was 0.5 ns, the wavelength of 532 nm, and. Three types of gold NPs were used to form the multi-NP clusters: gold spheres (50 and 60 nm), rods (25x75 nm) and shells (52 nm).

Although the tests have mainly only been done on cells of a Zebra-fish implanted with human prostate cancer samples, PNBs provide huge potential for the future of nanomedicine, as many doctors and scientists quote. Scientists believe that PNBs can provide a basis not only for diagnostics and therapeutics, but for biomedical research as well. The high sensitivity of the nanobubbles will bring opportunities of non-invasive, accurate imaging of tissues and cells. Delivery of drugs or impact will be more precise (i.e. to specific targeted cells) for many other types of cancer as well. The bubbles can also be controlled to a high standard, where a very particular intensity type of the laser must be used, which means they can be very particular about which cells to track down, especially now since they have the ability to use antibodies to detect certain cells. Plasmonic nanobubbles have many relations to future pathology. The light used in the procedure is naturally exposed to organisms anyway, and the fact that the lasers are short pulses, this method could become much cheaper than previous methods using inaccurate nanoparticles.

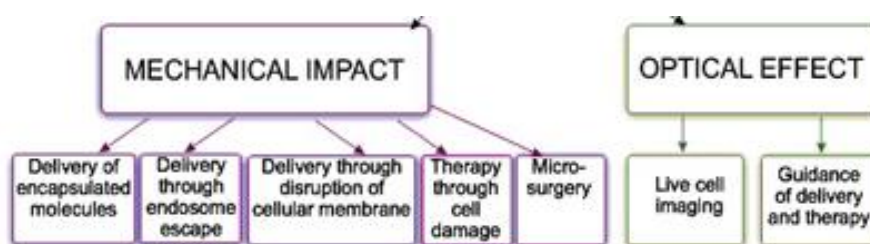


Figure 3B [11]

The other half of this figure (3B) shows the implications this technique has in general for future medicine. The method of using the first laser to detect the bubbles leads to developments in live cell imaging and a clearer guidance on delivering the therapy to the targets only. The structure and function of the nanobubble can imply improvements in these listed topics, including delivering therapy through disrupting the cell membrane and microsurgery. The adoption of this process would certainly reduce the deaths by prostate cancer in men and hopefully has a good ground for reducing the number of deaths by this cancer due to late diagnosis, or repetition of dangerous chemotherapy.

However although there are many advantages and hopes for this new idea, there are drawbacks and limitations all the same. Like many medical advances, they all tag along several ethical issues which must be acknowledged and addressed, by scientists, medical professionals and the patients themselves.

Probably the most obvious drawback is the question of availability and cost. Since this procedure has only been tested a few times, although it seems infallible, we may have to spend many years perfecting the technique ensuring that we reduce the risk of accidents or any unforeseen side effects before using it on people regularly. This could of course be costly, and even if we are able to openly use this technique on typical patients, it might be a very expensive form of treatment, which means the availability may be very sparse indeed. Although the use of the lasers would be relatively cheap compared to running continuous lasers in old nanoparticle theories for diagnosing the cancer, it may still be an economic drain to not only use the lasers at it's very particular attributes, but to manufacture the nanobubbles as well. The transient vapour bubble would need intense thermal energy to create and because it only lasts for a few nanoseconds, if the system failed it might be a big waste.

There may also be arguments that if a patient was being tested for prostate cancer and none of the plasmonic nanobubbles showed up as positive, it could either be a fault in which case the patient's cancer would continue to grow and be undetected, or it would be a waste of money, time and resources. The availability of this technique may mean more and more people want to be tested for the cancer, but pressure on the expense is too much which would lead to other problems too.

There may be social issues regarding testing the technique on certain other organisms. This test mentioned earlier used zebra fish which were injected with human prostate cancer. Scientists may have to carry out this test on other small organisms which may upset certain members of the community. It might also be a struggle to find willing human subjects with which to test it on, as for some cases in the past, side-effects are not always visible or predictable in preliminary testing.

A focal disadvantage which is unavoidable is that this technique is a relatively new idea and will need time for research and confirmation on its promise, which means the shocking statistics for prostate cancer in males will either remain stable or even increase due to other factors in the lifestyle of a human in the developed world. Yet once the technique has been perfected this may be a massive breakthrough in the history of medicine and a new, exciting find for the young field of nanomedicine.

Conclusion

The development of this new theory in using PNBs in theranostics of prostate cancer would be of huge benefit to patients. Early diagnosis and safe destruction of the cancer in two quick steps ensures efficient management of the disease and a significant reduction in the side effects brought on by current uses of treatment involving chemotherapy and other forms of radiation. Although much research and time must be put into this new idea, it already has a promising outlook and even though the drawbacks are as serious as those in other medical advances, these can be overcome through experimentation and perfection. The benefits will surely outweigh the expense of research. While this procedure is "in the making", other techniques such as using the carbon nanotubes as drug delivery systems may start to play a part in selectively destroying cancer cells to improve survival rates among sufferers.

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