‘The Silent disease’
An evaluation of recent scientific knowledge and treatment of Osteoporosis and possible future considerations of utilization of Nanomolecules for the universal disease

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PASS WITH MERIT

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Abstract:

This paper reviews the effects and causes of Osteoporosis, and evaluates recent research ideas, of how nanotechnology can assist the future treatment of the disease. It also conveys the notion, of how universal Osteoporosis has become, and how nanotechnology research is rapidly becoming more focused, and showing a great potential for bone diseases such as Osteoporosis. Moreover, the paper covers the development of how a multi-functional macromolecule; dendrimer can be utilized, by the aid of PEBBLE (Probes Encapsulated By Biologically Localized Embedding) nanosensors, in order to reduce the amount of bone fractures caused by Osteoporosis.

Introduction:

(1) Nanotechnology is a branch of engineering that has only recently begun to develop in leaps and bounds, dealing with molecules smaller than 100 nanometres. In 1959, Richard P. Feynman gave a talk on how molecular machines could be manufactured with Nano-precision. Following his talk, there have been many breakthroughs: Buckyball in 1985 and the first engineered protein in 1987. Nanotechnology in medicine had increased the chances and possibilities of improving the standard of living for people with singular and multiple diseases. Some of the types of nanotechnology that has been involved in medicine included the dendrimer. The dendrimer is an artificial polymer molecule. It is used for drug delivery, diagnostics and gene therapy. The reason the dendrimer is such an incredible macromolecule is due to the numerous voids that are within it, (2) which allows it to carry molecules condensed into their voids. Furthermore, the voids have a large surface area, which enables dendrimer to have great control and flexibility in its functions. Due to these characteristics, the molecules positioned in the dendrimer are only released when the suitable molecule is present.

In addition, to dendrimers, another breakthrough was the introduction of 'stents'. (3) A stent is an expandable wire that enables a blood vessel to be dilated, without it rupturing. First used in (3)1961, the stents were created by mire mesh or pleated fabric. However, some of the problems with using such material could result in thrombus forming causing resulting in a thrombosis. As a result, companies such as 'Advanced Bio-Prosthetic surface' have developed nitinol - a shape memory alloy. This material is effective as it remains flexible but springs back to maintain its shape.

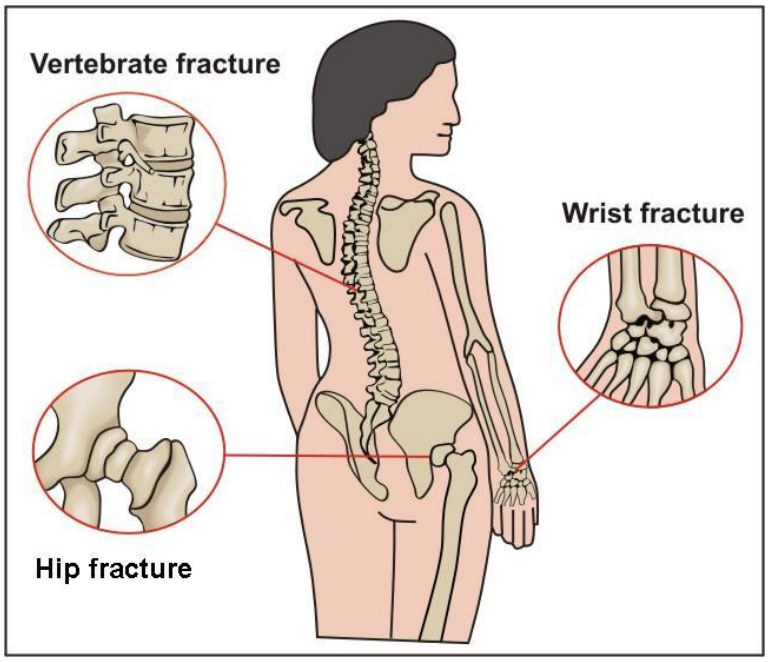
As well as this new material being discovered, the company have developed a manufacturing process by which the stent is created by sputter deposition: atom by atom manufacturing of the stent. During this process, there is a high success that the stent is free from contaminates and impurities which might cause problems such as; inflammations in the vessels when the stent is implanted. Another effective method, yet not currently in use, (4) in which bone cells are grown on scaffolds which are made of pure carbon Nanotubes. (4) These structures are extremely strong and firm. According to Laura Zanello from the University of California, these methods although not yet in use, could theoretically, with the aid of Nanotubes, could create a layer on the patient's bone cells, which could fit perfectly into a gap in a damaged bone. This method is effective because the body is unlikely to reject the Nanotubes-bone cells, since the carbon is bio-friendly and the bone would be grown from the patient's own cells.

However, even though this method may have a high potential, there is a diminutive amount of research on bone diseases like osteoporosis. Research linking bone diseases such as

Osteoporosis and Nanotechnology is still in its infancy but there are many people with a high risk of developing the disease.

Osteoporosis; (5) known as porous bones; is a disease where any bone in the body becomes weak by losing its mineral contents and mass, this eventually leads to fractures and even disability. The most common bones affected are: (5) the hip bone and spine as they are under constant pressure and stress as shown in Figure 1 (6).

Figure 1:



Many of these fractures result in: reduction in height, inability to move and disability and many cases demand immediate hospitalization and even surgery, (7) as shown in Figure 2. (5)Many people are often surprised when they are diagnosed with osteoporosis, as loss of bone mass happens over a long period of time without the person's knowledge.

Figure 2:

People affected each year by osteoporosis:	**Number of people affected by osteoporosis:**
Fractures from osteoporosis	1500,000
Hospitalisation	500,000
Trips to the emergency room	800,000
Visits to the doctor's office	2600,000
People placed in nursing homes	180,000

It is estimated that:

- 10 million people over the age of 50 in the US have osteoporosis – 80% are women (5)
- In the UK there are 3,000,000 people with osteoporosis and as a result over 230,000 osteoporosis related fractures each year (8)
- One in two women will suffer osteoporosis-related fractures (5)
- One in four men will suffer osteoporosis-related fractures (5)
- 20% of senior citizens who suffer a hip fracture die within a year (7)

(7) Richard H. Carmona, M.D., M.P.H., FACS (Surgeon General) alleged that: ' Due primarily to the aging of the population and the previous lack of focus on bone health, the number of hip fractures in the United States could double or even triple by the year 2020.'

Osteoporosis can affect both genders. (9)There are many causes of osteoporosis in both women and men but the most prominent is hormone imbalance. Women in particular, have an accelerated bone loss after menopause as they have a rapid drop in oestrogen levels. Oestrogen is known as the hormone that aids in regulating a woman's reproductive cycle, as well as; (10) encouraging the storage of calcium, that aids in sustaining the strength of the bones. Women usually have more oestrogen than men, before becoming menopausal, therefore they are more likely to experience more bone loss due to the decrease in oestrogen levels. Men also need oestrogen for healthy bones, however, they acquire the hormone oestrogen after converting the hormone testosterone into oestrogen, and consequently**,** osteoporosis occurs in men when not enough testosterone is being converted into oestrogen. (11) Therefore when diagnosing men with Osteoporosis, Doctors would evaluate their testosterone levels rather than their oestrogen.

(9) **Other causes of Osteoporosis include:**

- Aging
- Lack of calcium
- Vitamin D deficiency
- Thyroid conditions
- Inactive lifestyles
- Smoking
- Drinking

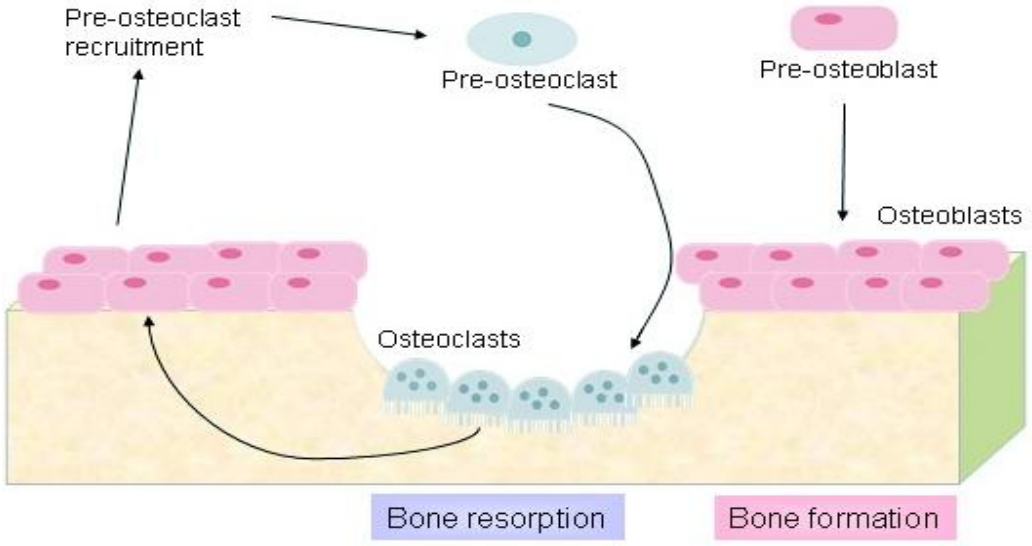
(11)Despite the above causes, women are more at risk than men for getting the disease osteoporosis due to the large shift in oestrogen levels after menopause.

Osteoporosis develops when; the rate of bone loss is greater than the rate of replacement. Although this process is gradual, the decrease in estrogens levels during menopause accelerates bone lose dramatically.

(12) Bone is made up of collagen, consisting of two components: a protein; which provide the soft framework, and calcium phosphate; which hardens the framework. Nevertheless, the bones undergo dramatic remodelling continuously, and this is due to the calcium levels in the blood.

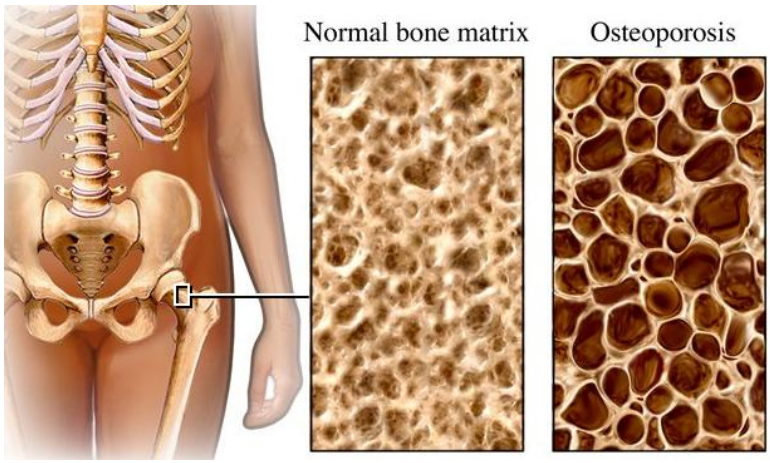
When calcium levels in the blood fall too low, cells called osteoclasts, dissolve the bone required amount of bone tissue. This allows calcium to enter the blood stream to control various factors e.g. heart rate. In response to a decrease in bone mass, cells called osteoblast, replace the areas of low bone mass with collagen and phosphates, as shown in Figure 3 (13).

Figure 3:



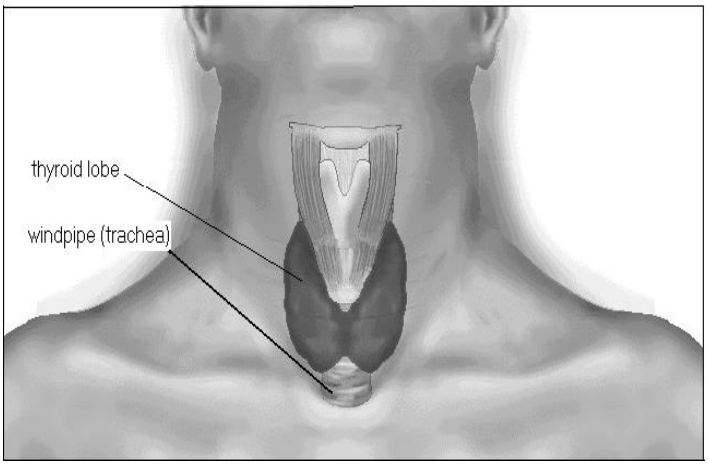
(18) Bone density decrease, after the ages of 35, as the rate of formation is lower than the rate of removal, leading to 2% of bone mass being lost per year. When there is a significant difference between the rate of formation and the rate of resorption, it leads to weak bone, therefore causing osteoporosis, as shown in figure 4 (12).

Figure 4:



(14)The thyroid gland is responsible for making and releasing hormones that control and regulate the body's metabolic rate. The gland also synthesises a hormone that helps to maintain the amount of calcium in the blood, (14) and is situated at the base of the neck, as shown in figure 5 (15).

Figure 5:



(15) Some of the hormones that the thyroid gland synthesises are: T3 (Triiodothyronin); T4 (Thyroxin); calcitonin. The T4 and T3 help to maintain the metabolic rate. However, calcitonin, works in partnership with another hormone called parathyroid hormone located in the parathyroid gland, to regulate the amount of calcium in the blood.

(15) Calcitonin lowers the calcium in the blood, by stimulating the bones to preserve the calcium and the kidneys to secret it out through the urine. Whereas the parathyroid hormone stimulates the release of calcium from the bone into the blood, enabling the kidneys to reabsorb the calcium, before it is lost in the urine. (15) (16) Too much production of calicitonin will put a strain on the thyroid gland causing it to go into hyperthyroidism, which can initiate weight loss.

(16) Other symptoms include:

- Weight loss
- Rapid heartbeat
- Tremor
- Excessive sweating
- Heat intolerance
- Anxiety
- Muscle weakness
- Goitre
- Irregular periods

(17)Osteoporosis can have a devastating effect on individuals' living conditions. Suffering osteoporosis brings in a colossal amount of effects on an individuals' body. The first consequence of osteoporosis is spinal column misalignment; in many cases the spinal column become so fragile that it prevents an individual to stand upright and have a proper posture. (17) Another common bone affected is the hip; this can have life threatening consequences as individuals can experience other complications such as blood clots, pressure sores and pneumonia. Osteoporosis prohibits an individual to carry out simple activities such as climbing the stairs; it creates immense pain in the body to those suffering from the disease. Although, common research shows that Osteoporosis mostly occurs in women in their post- menopausal stage, a recent case study shows that (17) osteoporosis can also affect women early in their 20's. 'Salima Ladak Kachra from Canada sustained four compression fractures in her spine when examined with an X-ray, after she had slipped and fallen. As well as sustaining compression fractures, she also decreased in height by one inch and had her waist increase by four inches. Even after seeing few physicians about her back pains, they were unable to detect that she had osteoporosis. In spite of this, her family's medical history showed that many of her close relatives suffered from osteoporosis. As well as being genetically prone to the disease, she also had other common factors that may have contributed**:** diminutive body; Asian descent; low calcium intake as she was lactose and tolerant; irregular menstrual cycle. (17) After seeking a new family doctor, who did a comprehensive blood and diagnostic tests, it revealed that she had Hyperprolactinemia. Hyperprolactinemia is a condition by where it prevents the patient from attaining their peak bone mass and therefore resulting in the patient having thin bones. After further examinations of her bone density, it revealed that she had severe (17) Osteopenia in both her spin and femur regions, which required immediate measure to be taken.

Discussion:

(19)Osteoporosis has been considered to be one of the 'silent diseases' as it is unnoticed until after a fracture occurs. This therefore results in a later diagnosis of the disease. A way for diagnosing Osteoporosis is the involvement of physical examinations as well as measuring bone mass. Bone mass measurements are usually taken from the common areas where bone fractures occur; spine, wrists and hip. Bone mass measurements are taken through performing a bone material density test to the sufferer. To perform a bone material density test, an ultrasound device is needed. This modern medical instrument transmits sound waves to the sufferer's heel and then the bone density results are analysed. However, a major disadvantage is that results show poor correlation hence this method of diagnosis is often considered inaccurate. (20) Consequently, an accurate method for the diagnosis of Osteoporosis is instead used; DEXA scan. DEXA scan also known as The Dual Energy X-ray Absorptiometry is another type of bone density test which examines bone density mainly at the spine and hip.

Modern treatments are used to either ease the pain caused by Osteoporosis and to prevent further bone fractures. One of the treatments is through the use of medications: drugs such as Bisphosphonates. (21)(22) Bisphosphonates reduce bone loss therefore consequently increases bone strength and results in abridged risk of fractures. This class of drugs function by restraining the formation of osteoclasts – these are multinuclear cells which are responsible for the breakdown

of bones. Although this drug has been used since 19th century, it has many underlying side-effects. (21)One of the main side-effects in using Bisphosphonate includes the risk of osteonecrosis.

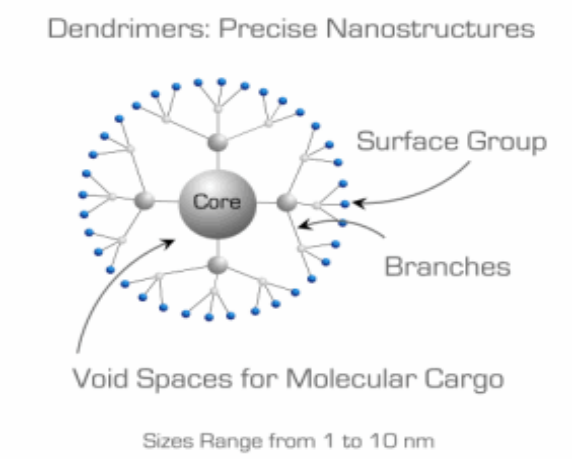
Although, there are modern treatments for osteoporosis, the use of nanotechnology could potentially increase and allow the treatment to be more efficient and effective. The research suggests that the use of certain nanomolecules i.e. dendrimers, PEBBLES with the aid of Calcitonin, can possibly diminish the effects of osteoporosis without continuous drug prescription**.**

Dendrimers:

(23)Dendrimers were first discovered in the early 1980's by Donald Tomalia. These are polymeric macromolecules composed of numerous branched monomers radically originating from a central core as shown in **figure 4.** Due to the large amounts of voids in a dendrimer, it has a large surface area. Since there are many types of dendrimers, there can be a variety of molecules that a dendrimer can contain. However, all the dendrimers contain a (24) porphyrin core and carbazole-based chromophores as branches. (23) **Figure 6** shows a labelled dendrimer molecule. (25) There are two methods to create a dendrimer: the divergent method and the convergent method.

(26) In the divergent method, the dendrimer is created when the core molecule reacts with monomer molecules containing one reactive and two dormant groups. This produces the first generation of dendrimers. Following this, the new external boundary of the molecule is activated for the reaction and attachments of more monomer molecules. After many attachments of monomers, layer by layer a dendrimer is created. However due to many side reactions occurring during the synthesis of a dendrimer and incomplete reactions of the end groups, a new method was developed: the convergent method.

Figure 6:



In the convergent method instead of the dendrimer being synthesised from the core outwards, in this method the dendrimer would be created stepwise starting from the end groups and progressing inwards. An advantage of this method is that it makes the purification of perfect dendrimer simple. (26) Due to their large surface areas and unique properties, dendrimers hold the possibility to be used as drug carriers in the large amount of voids. They can do so by

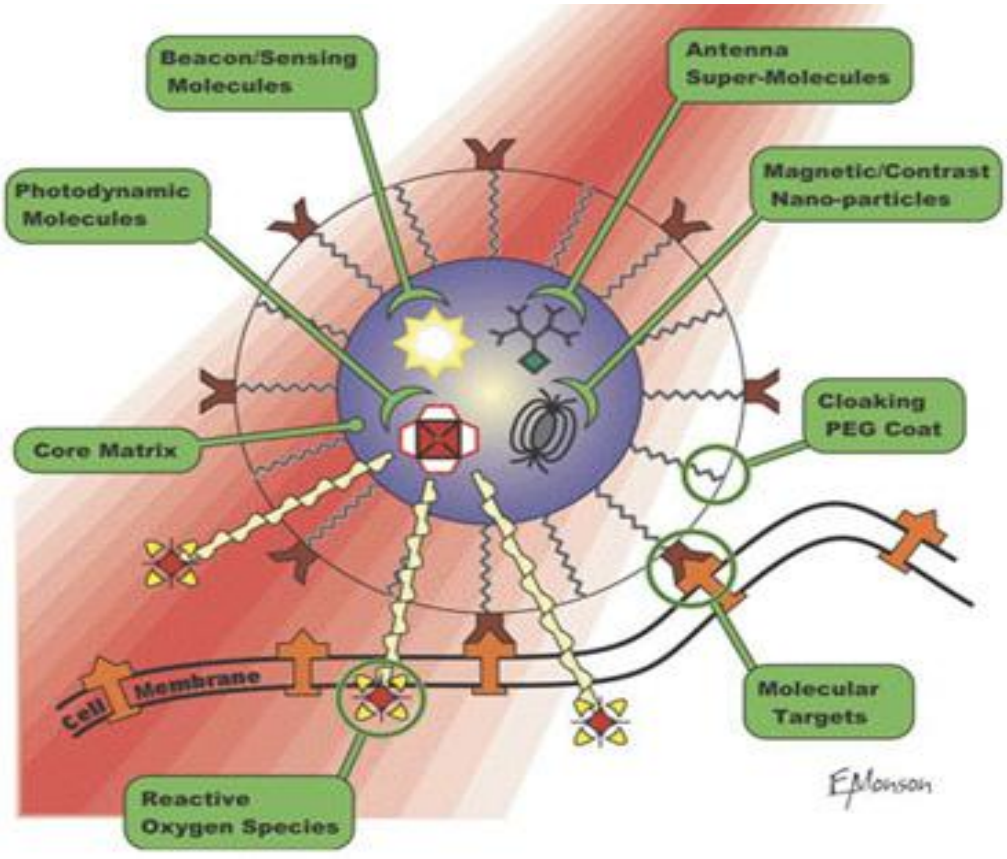
encapsulating drugs within the dendritic structure, or by inter-acting with drugs at their boundaries. As well as a future breakthrough for drug delivery, (27) Dendrimers can also be used in gene therapy , by carrying genes into cells as part of a virus that has been disillusioned due to the lack of getting genes into enough cells to make therapeutic differences. Moreover, the amine groups projecting on the dendrimeric's surface helps the dendrimer to by-pass the body's immune system, which allows effective drug therapies.

PEBBLES (Probes Encapsulated By Biologically Localized Embedding)**:**

(28)Discovered by Raoul Kopelman, PEBBLES are probe encapsulated by biologically localized embedding sensors, ranging from 20 to 200nm in size, they consist of sol-gel matrix integrated with a fluorescent dye. PEBBLES have been developed so that they could be used for the analysis of chemicals such as calcium, potassium, glucose and oxygen. PEBBLES have the ability of being carriers for varieties of agents on their surfaces. **Figure 7** shows the surface of a PEBBLE molecule with labelled are areas.

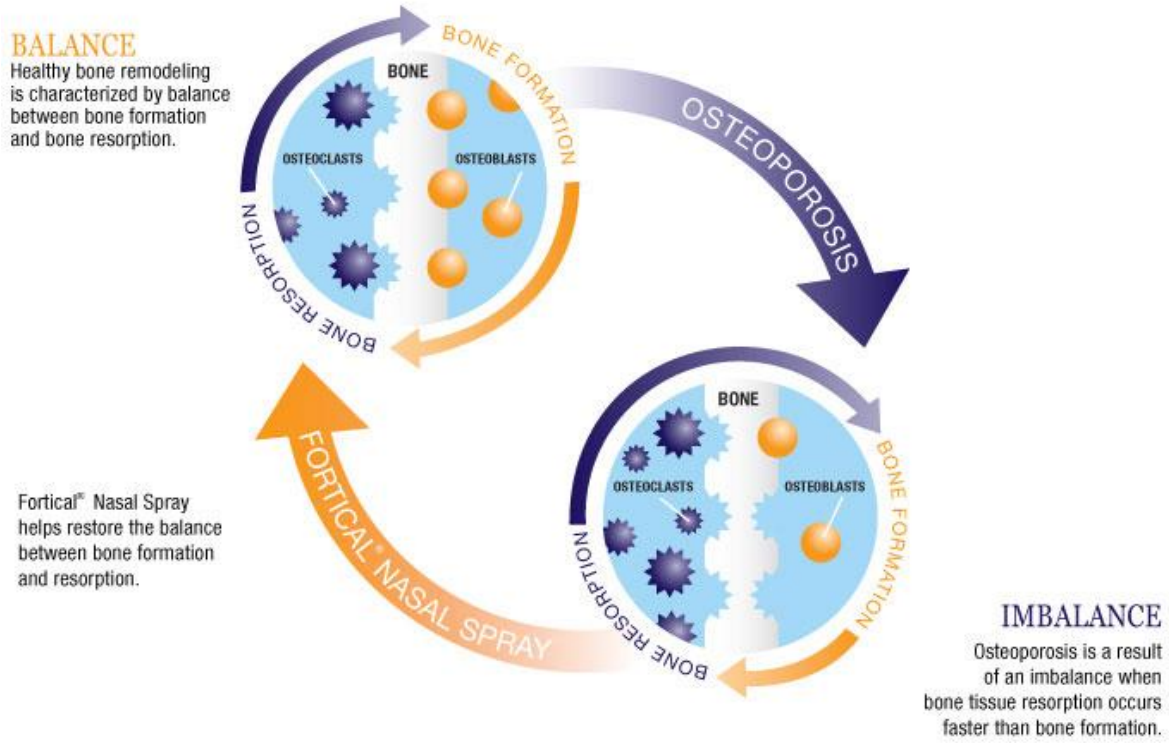
PEBBLES being multi-functional can be used for many medical purposes; (29) one of which is to treat cancer. This can be done by placing one target molecule on the surface of the pebble that could guide the pebble to the tumor. (29) As well as this 'guiding agent' there are other two functions that could be placed on the PEBBLES surface that could be combined in a single tiny polymer sphere to make a cure against cancer. However another functional step must be carried out to make the PEBBLE successful in its role. (29) This functional step is that each PEBBLE carries a photo catalyst and when stimulated by a light source through a micrometer sized fiber optic probe inserted into the skull, the photo catalyst converts oxygen into a single stat, which bleaches and destroys the surrounding cells. (29) This experiment was conducted by Raoul Kopelman, Ph.D. at the University of Michigan, on rats with the 9L-gliosarcoma cancer, where PEBBLES were injected into the brains of each rat. It has not been tested on humans but results from the experiment were promising. (29)

Figure 7:



(30)Calcitonin-salmon is a hormone that we wish to insert into the voids of the dendrimer. Calcitonin is produced by the thyroid glands in a salmon and prevents the osteoclasts from functioning and promotes formation of osteoclasts. (31) The reason we wish to use calcitonin, is because it has been proven to increase bone density and strength in women with osteoporosis**.** (32) Calcitonin assists in re-establishing the balance between bone formation and bone resorption, as shown in figure 8 (32). Present methods of administering Calcitonin are either injecting it under the skin into a muscle or to administrate the drug through the nostrils by a nasal spray.

Figure 8:



Although this hormone drug is foreign to the body, it has some mild side effects. (31) These include: nausea with or with the absence of vomiting in 10% of the patients; redness of skin at the site where the drug was injected; flushing and skin rash; runny nose; nose bleeding; headaches.

Using all the above research, we believe that the hormone synthesized drug; calcitonin, when (29) immobilized can be positioned in the multiple voids of the dendrimer. The dendrimer can then be attached to the molecule target areas of the PEBBLES; this will then act as the target molecule which will guide the PEBBLE to the thyroid gland and to the osteoclast cells can be found. Once situated on the gland, the PEBBLE can stimulate the other dendrimers on the surface of the PEBBLE to release the other (29) mobile calcitonin molecules from their voids, which will prevent the osteoclast calls from functioning. This will then force the osteoclast cells to group together to assist the PEBBLE to (29) release a toxin from its surface in order to destroy osteoclast cells specifically. The osteoclast cells are destroyed by a photo catalyst when stimulated by a light

source. (29) The attachment of another agent on the surface of the PEBBLE would aid in regulating and visualizing a significant change in the quantities of osteoclast cells. (12) The reduction in osteoclast cells would prevent the bone from becoming weak and causing fractures.

Conclusion:

Nanotechnology has portrayed great ability and potential to be used in the treatment of osteoporosis and enable the effects of the disease to be reduced. Our reading implies that rather than using a technique for normal drug delivery to aid in osteoporosis, we can gain a deep understanding of vertebrae anatomy via nanomolecules such as; dendrimers and PEBBLES, to enable a successful breakthrough. Moreover, since recent studies have shown how many people are unaware about the disease thus are often surprised when diagnosed, raising awareness is also one of our main objectives in order to lessen the millions of people suffering fractures without the knowledge that they had the disease beforehand.

Although we have researched the application of dendrimers and PEBBLES as a combined drug, we have not yet deduced a method of permanently attaching the PEBBLES to the thyroid gland for it to regularly release the hormone, Calcitonin. Furthermore, extended studies need to be made to confirm if dendrimer molecules can be positioned on the molecule target of the PEBBLE's surface. Therefore, we would like to see further research on whether it would be possible for the attachment of the PEBBLES to the thyroid gland, as well additional research as to whether or not nanomolecule such as dendrimers can be installed onto the surface of the PEBBLE. In addition, from the experiment of injecting PEBBLES into the brain of rats, it showed the potential success of nanotechnology. However, as this research is still in its early days, studies need to be carried out on higher primates to verify that the research on PEBBLES is conveyable. Moreover, we are irresolute as to how the intake of the combined nanomolecule in the neck area could take place.

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