A Biomaterial Solution for Osteoporosis-Related Fractures

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Group 16 - Stage 2 Report

Problem Statement

Currently, the extent of biomaterial treatments for osteoporosis-related injuries is limited to preventative measures that can be implemented after a diagnosis of osteoporosis or osteopenia. After a fracture has occurred, most likely due to a fall or other stress, mechanical solutions such as screws and plates are utilized. However, in the case of osteoporosis, current mechanical fixation methods do not address the underlying problem of decreased native bone density. As a result, reinjury often occurs due to weakness of the osteoporotic bone or the bone-implant interface. As will be discussed, there is a need for a biomaterial solution for repairing such fractures that is biocompatible, biodegradabile, and possesses the required mechanical characteristics.

Background

Osteoporosis is a disease in which bone density decreases over time. This disease affects all sexes at a varying rate. Low bone density in osteoporotic patients makes them prone to fractures that are caused by low impact falls. After treatment, the recovery process for an osteoporotic patient is comparatively longer than that of a person with healthy bone density. To minimize the need for treatment and recovery, osteoporotic patients are introduced to preventive solutions such as high calcium diets and fall prevention techniques.

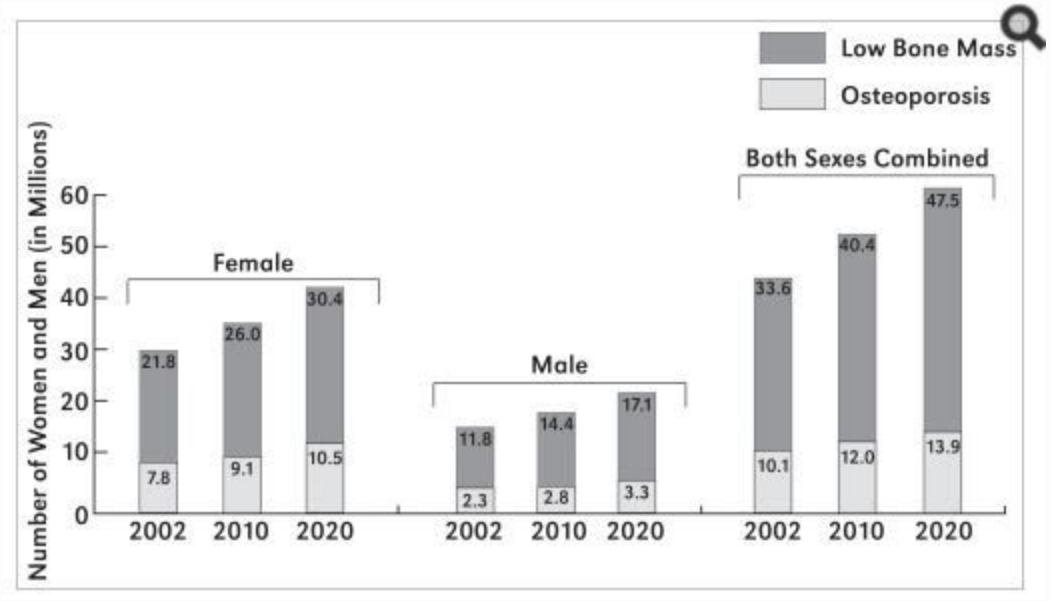

Figure 1: Projected Prevalence of Osteoporosis and/or Low Bone Mass of the Hip in Women, Men, and Both Sexes, 50 Years of Age or Older, *Bone Health and Osteoporosis: A Report of the Surgeon General*.

In *Figure 1*, we can see that, in 2020, an estimated 13.9 million people from both sexes and above the age of 50 are projected to suffer from osteoporosis. Women above the age of 50 are more prone to osteoporosis than men. According to *Archives of Osteoporosis,* fragility fractures secondary to osteoporosis are highly prevalent, with about 9 million cases yearly worldwide. The three requirements for the biomaterials used to treat these fractures are biocompatibility, biodegradability, and mechanical strength.

In stage one report, we discussed the impact and limitations of the pre-existing solutions to treat fragility fractures. Polymethylmethacrylate (PMMA), a kind of bone cement, is used to ensure the placement of mechanical devices and also treat compression fractures. PMMA is biocompatible, but the monomer formed during polymerization (MMA) leads to the deterioration of healthy cells. PMMA does not have adhesive properties, as it relies on an interlocking mechanism between the irregular bone and the prosthesis. In patients with low bone density, PMMA may cause the prosthesis to slip. An alternative to PMMA is calcium phosphate cements (CPCs). According to *Developments and Applications of Calcium Phosphate Bone Cements*, the degradation products formed from CPCs facilitates the growth of new bones and hence boosts osteoconductivity. The mechanical strength of CPCS is inversely proportional to its microporosity. Though microporosity is an important property for osteoconductivity, it affects the cement's ability to perform load-bearing applications. This can be easily remedied by adding fibers like trimethyl chitosan (TMC) to make the cement tougher. This report will further discuss the properties of CPCs and how they are better suited to treat fragility fractures.

Proposed Solution

Calcium phosphate cements (CPCs) are biomaterials used in an emerging method of bone repair that can be very successful in treating osteoporotic-related injuries. Prior to the discovery of CPCs, organic bone cements were used to repair bone injuries. However, these cements had major biocompatibility issues, resulting in irritation and inflammation. CPCs were initially discovered in the early 1980's by scientists LeGeros, Brown, and Chow. CPCs proved to be a viable alternative to the organic bone cements used before. Calcium phosphate cements consist of a mix of calcium phosphate powders which form a paste-like substance when combined with a liquid. This paste can then be applied directly to the bone at the site of a fracture. CPCs solidify through a dissolution-precipitation reaction, which can be triggered by body temperature alone. Therefore, CPCs self-set and solidify *in situ*, forming a support around the bone and fracture.

Calcium phosphate cements' ability to self-set/solidify *in situ* and high flowability lead to a critical advantage of the biomaterial: injectability. Typical bone graft operations require large and relatively complex open surgeries. However, CPCs can be injected at the site of bone fracture with minimally invasive procedures, resulting in reduced pain, easier recovery, and a smaller surgical scar. Therefore, calcium phosphate cements are a more safe method of

bone-repair. Additionally, the ability of CPCs to be injected at the site of injury also allows for CPCs to be coupled with drugs or biological molecules and serve as a carrier.

In order for a biomaterial to function correctly inside the body, it is vital that it possesses a very specific set of properties. For bone-repair in specific, it is important to understand a material's biocompatibility, bioactivity, and biodegradability. Because bone cements are injected into the body, it is important that they are biocompatible, and they do not have a negative interaction with surrounding living tissues. After the self-setting reaction is complete, calcium phosphate cements are composed of apatite (HA/CDHA) and brushite (DCPD). These substances are known to be biocompatible. Studies determined that CPCs are also bioactive materials, and they bind to bone without the formation of additional tissue. It is also key that as new bone forms, the bone cement biomaterial degrades. In order to achieve a sufficient rate of degradation, CPCs can be coupled with PLGA. PLGA degrades rapidly when it encounters water, producing lactic acid and glycolic acid. These acids then enhance the degradation of CPCs, as CPCs degrade by acid dissolution.

One of the primary advantages of utilizing CPCs for repairing fractures in osteoporotic bone is their osteoinductive properties. As mentioned previously, the main complication associated with internal fixation of fractured osteoporotic bone is failure of the bone itself rather than implant failure. To remedy this, the metal implants commonly used to fixate major fractures can be augmented with CPCs to induce osteogenesis at the site of fracture. This will strengthen the damaged bone tissue and reduce reinjury risk. In the same vein, CPCs serve as excellent scaffolds on which new bone tissue can grow. The high porosity of this bioceramic enables extracellular proteins to be easily absorbed onto the surface. These proteins trigger signaling pathways which lead to cell reproduction and new tissue generation. This can be especially useful in strengthening the bone-implant interface when large implants are used as fixation devices. Augmenting fixation devices with CPCs will trigger increased osteogenesis at the bone-device interface, reducing the previously high risk of bone failure. The osteogenic properties of CPCs can be further improved by seeding with various stem cell lines. Relatively new studies have shown that seeding CPCs with human induced pluripotent stem cells (hiPSC) and human embryonic stem cells (hESC) increased osteogenesis 2-3 times relative to non-seeded controls (*Wang et al.,* 2014). Seeding

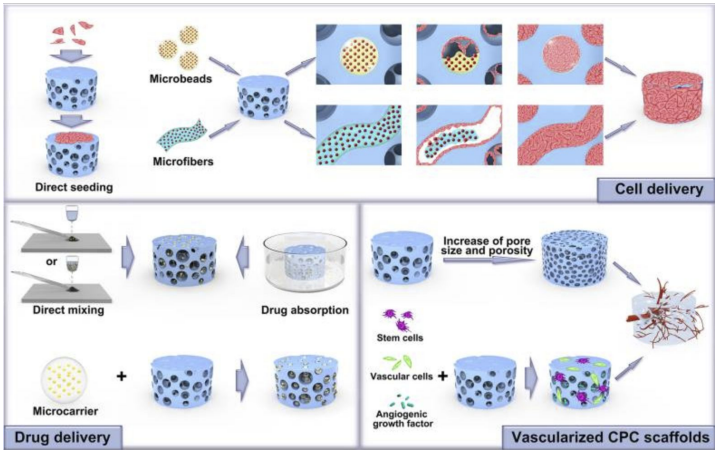

Figure 2: Versatility of CPCs as a bioreactive therapy (*Eliaz, et al.,* 2017).

CPCs with human bone marrow mesenchymal stem cells (hBMSCs) is also a very promising method of increasing osteogenesis.

Another common complication with bone fixation in general, and especially fixation of osteoporotic bone, is stress shielding. When a fixation device such as a metal rod or artificial hip replacement is introduced, much of the stress is transferred from native bone structures to the artificial implant. As a result, native bone does not get sufficient mechanical stimulus for continued remodeling and reproduction of tissue, leading to a decrease in bone density. This problem is amplified in the case of already weakened native bone seen in osteoporosis patients. In addition to stem cell seeding techniques, prevascularization of CPCs has been shown to increase osteogenesis rates (*Wang et al.,* 2014). While fixation with polymethyl methacrylate is a well-documented and effective mode of providing immediate, strong fixation of the interface between implant and native bone (*Vaishya, Raju, et al.,* 2013), it does not address the underlying problem of weak native bone tissue surrounding the implant in osteoporosis patients. This is the main advantage of CPCs, so methods of improving osteogenesis rates are paramount in creating more expedient clinical improvement.

While many methods of improving biofunctionalization of CPCs, such as stem cell seeding techniques, have been reviewed in the literature, a novel approach to widening the scope of CPC uses lies in improving the mechanical properties of the bioceramic itself. Currently, CPCs are primarily used for repairing cranial and maxillo-facial fractures (i.e. fractures on non-load bearing structures) due to their low mechanical strength (*Vaishya, Raju, et al.,* 2013). Potential approaches to increasing mechanical strength of CPCs include tailoring the porosity and percent crystallinity of the ceramic. While reducing porosity would increase the tensile strength of the CPCs, it would significantly reduce the tissue scaffold and osteogenesis benefits of the bioceramic, which are their primary advantages over other cements like PMMA in treating osteoporotic fractures. Another potential route of increasing mechanical strength without altering the CPC itself is creating a CPC-fiber composite. Introducing chitosan fiber into CPC to form a composite has been shown to significantly increase the compressive strength of the CPC bioceramic (*Lian, Q., et al.,* 2008). This chitosan fiber augmentation of CPCs is also thought to improve native bone repair processes and expediting the healing process. This versatility makes CPCs a very promising solution for improving fixation of historically unstable osteoporotic fractures.

Models for Testing and Evaluation for Success

In vitro testing for any bone cement implant is necessary according to the International Organization for Standardization (ISO) in order to assess the possible angiogenic and osteogenic repercussions, oxidative stress, and tissue inflammation (Albulescu et al, 2019). In general, the chemical nature of the CPC's, which are of the chemical formula Ca10(PO4)6(OH)2 or β-TCP,

Ca3(PO4)2, are very similar to bone mineral (hydroxyapatite) , which has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Due to both CPCs and bone mineral possessing ceramic quality, there is a high probability for nano-debris, which is produced via degradation (Albulescu et al, 2019). To test for the probability and amount of nano-debris, an in vitro cytotoxicity assay is used, as recommended by ISO 10993-5: 2009 (Albulescu et al, 2019). Cytotoxicity assays are designed by exposing CPC cells to osteoblasts using two or more cell lines. Using multiple cell lines allows the assay to monitor all the possible downsides of not only debris, but the other parameters mentioned previously. In order to design a cell line to assess for the risk of nano debris, or nano-toxicity, the particle size must be determined either through microscopy, spectroscopy, or another method (Jones and Grainger, 2010). Particle size determination is vital because grain size can be a determinant of damage that can be done by nano debris. In general, the most common cell lines to determine cytotoxicity include, but are not limited to, an assay that can determine if bone-marrow monocytes can uptake nanoparticles, if nanoparticles bind to the surface of osteoblasts and start deposition, and if nanoparticles cause adverse reactions through surface binding.

If multiple assays determine that the proposed CPCs will not cause adverse biological reactions or decrease health of osteoporotic bone, small animal models can be then employed. In general, animal models for any bone implant with a biomaterial are chosen based upon whether the model can produce a similar physiological and pathological response to a human. Due to the low cost and accessibility, mice models have become most common for testing bone cements, such as CPCs. If the specific bone cement has never been implanted in the mouse model before, a reference material, with a known biological effect, will often be planted in another location (Shi, 2011). Both materials will be placed between the derma and scapular to assess toxicity before planting into the bone (Shi, 2011). Histology of the dermal tissue will be evaluated qualitatively. In small animals, a successful small animal will not have any adverse reactions to the biomaterial. The success of CPCs for osteoporosis in small animals will be a seamless dermal implantation that is not followed by toxin deposits, unwanted polymerization, or other adverse effects.

After a small animal model has been seen as successful, a large animal will be used to assess if the CPCs can be used on osteporotic bone. In past studies, dogs have been favored for large animal models given that their bones are able to hand the ISO recommended implants size of 4 mm or less (Pearce et al, 2007). An incision is created further into the skin than the rat model and placed near the scapula. In the rat model, initial qualitative assessment is key. First, the dog model must be evaluated for any deposit or toxins to the bone site. In addition, any damage or development of new blood vessels is assessed (Pearce et al, 2007). No osteoporotic conditions are onset in the dog model as healthy bone must be tested first. If healthy bone is damaged, osteoporotic bone will be exponentially worse. Osteoporosis conditions can then be mimicked in older dog models. Success in these large animal models will be viewed as a decrease in fracture and refracture occurrence. In addition, no further propagation of osteoporotic cracks should occur with the application of CPCs.

Before the CPCs can move onto human trials, the path of cement deposition must be theorized and modeled, specifically deposition onto the bone surface. Therefore, when the CPCs are

implanted, the correct amount of material will be used based on the area of bone needing to be covered. Oftentimes, bone cement deposition is modeled using the well-known Langmuir model (Shi, 2011). After all three levels of testing are deemed a success, phase I and II human trials can begin.

Overall, any biomaterial that will be in contact with human bone is prone to risk. For the process of developing a bone cement material, the FDA outlines thorough development and testing guidelines that must be followed, as seen in Figure 3.

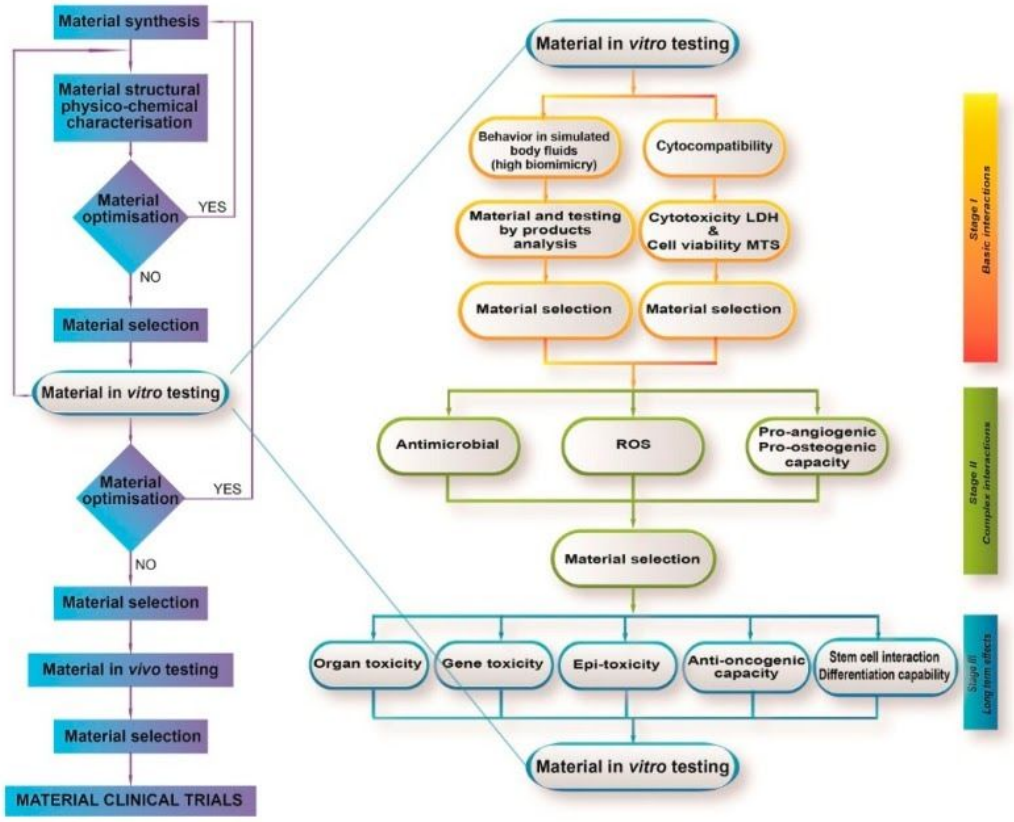

Figure 3: FDA Guidelines for developing bone cement biomaterial with calcium phosphate base (Albulescu et al, 2019)

Limitations

While calcium phosphate cements are a viable solution for osteoporosis, certain limitations exist in the material itself. Some of the mechanical limitations, when examined under the scope of bulk properties, are that these ceramics can be brittle and crack under a high amount of strain. These limitations are particularly seen in application of material to joints. Joints within the body are responsible for handling mechanical transfer of loads, which can put a lot of strain on materials placed within that area. To mitigate this, a more ductile, tougher material such as metal can be used as the load bearing materials while CPC can be used to create a biocompatible interface.

Another limitation of CPC is that prevascularization is required for proper osteoinductivity. In order for bone to generate properly, the development of new blood vessels within the bone is necessary. However, this occurs too slowly on its own and the new cells die because of lack of oxygen. To solve this, stem cells are used to create prevascularization, but the methods for acquiring those stem cells are controversial. To mitigate this limitation in the material, it might be necessary to find a cross functional solution (supplement the calcium phosphate cement with some sort of localized drug that would encourage faster angiogenesis).

Future Steps

CPCs offer a solution to treating osteoporotic bone in that they are osteoinductive, injectable, and biodegradable, among other desirable qualities as outlined above. One of the main areas that still needs to be improved upon is increasing the rate of osteogenesis as a means of fixing osteoporosis at the root of the problem rather than simply covering the issue. As touched on above, one of the most promising directions to achieve this goal is seeding the CPC with stem cells. However, this method is controversial in the source of stem cell harvest. Another interesting possibility for future work is a drug eluting implant that would release a drug to either induce osteogenesis or prevent bone breakdown

Osteogenesis is the cycle by which new bone is formed from a cartilage precursor. Osteoclasts are cells that play an important role in this cycle, in that they destroy bone tissue to form a cavity for bone marrow to fill (Gilbert, 2000). However, if the osteoclasts are not tightly regulated then too much bone will be destroyed and osteoporosis will result. Drugs that inhibit the action of osteoclasts help stop the destruction of bone. Bisphosphonates are one such class of drug that shows promise in this area. They are taken in by osteoclasts and prevent their function, slowing or stopping the breakdown of bone. Studies have proven their efficacy but in clinical settings they have seen limited success due to poor compliance with dosing (Lewiecki, 2010). This makes bisphosphonates excellent candidates for a drug eluting solution as it takes the human factor out of the treatment, so forgetting to take the drug or taking it at improper times would not be an issue.

Another option for the treatment of osteoporosis is drugs that induce an increase in bone generation, rather than simply reducing the rate of breakdown. This could allow a patient to regrow already lost bone, restoring strength and removing the need for further therapy in the future. One method of doing this is a combination of teriparatide and denosumab. Teriparatide is the only available drug that causes an increase in bone tissue regeneration, but some studies have shown that it increases bone resorption and the activity of osteoclasts a proportional amount (Lindsay, 2016). Combining the teriparatide with a drug that interferes with osteoblasts, denosumab, has been shown to increase bone tissue generation while limiting resorptive activity

(Cosman, 2019). Teriparatide also has the clinical limitation of only being allowed for use in a 24 month period after treatment begins. This is to protect against the possibility of bone generation going out of control, leading to cancer. Because of this limited treatment window, proper dosing and adherence to a dosing regimen are absolutely paramount. As seen with bisphosphonates, this could make teriparatide and denosumab prime candidates for implementation into a drug eluting solution, as it would ensure proper dosing and adherence through removal of the human aspect of drug therapy.

Conclusion

Osteoporosis is the leading cause of fragility fracture due to the low bone density seen in osteoporotic patients. Treatment methods used to treat normal fractures are not very effective in the case of ostioporotic patients. The frailty of the bone, sometimes doesn't provide adequate mechanical support for bone cements and other biomedical devices. In this paper, we have proposed the use of CPCs to treat fractures in osteoporotic patients. The biocompatibility, biodegradability, and adequate mechanical strength of the cement theoretically makes it a good choice for further testing. The porosity of CPCs makes it biocompatible, but decreases its load bearing ability. This is one limitation of CPCs that leads us to the theoretical conclusion that it is not suitable to be used in joints. In the future, we can take steps to create a treatment that integrates osteogenesis inducing drugs and chemically strengthened CPCs. Despite the brilliant advances made to treat osteoporosis, new research is needed to enhance the effectiveness of the treatment.

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