Localized therapy for wear particle mediated osteolysis via cement interfaces

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David Glover

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Immunoengineering

Localized therapy for wear particle mediated osteolysis via cement interfaces

Overview

Metal-on-metal implants such as knees or hips release wear particles that interact with the immune system leading to a Type IV lymphocyte mediated immune reaction. This immune reaction may lead to degradation of the bone or the cement used as the bone-prosthetic interface used to hold the implant in place. There are over 1 million titanium hip replacement surgeries performed annually in the US, with 10-20% requiring revision [1]. The resorption of bone by osteoclasts is triggered by phagocytosis of wear particles by macrophages, and triggers osteoclast differentiation and later osteolysis of bone or cement material. In this proposal, a system is presented where a therapy is encapsulated into microspheres and embedded into the cement interface to combat the immune response triggered degradation. These microspheres contain Osteoprotegerin (OPG), and are released as the cement is degraded and interferes with osteoclast differentiation. Receptor activated nuclear factor-kappa B ligand (RANK-L) is a key regulator of osteoclast differentiation from precursor cells, but is also a regulator of osteoclast activation into its mature differentiated state. Osteoprotegerin (OPG) is a RANK-L inhibitor which has been shown to be effective when administered intravenously in preventing osteolysis [2-4]. By encapsulating an osteolytic inhibitor into the cement interface, as the cement degrades there is produced a localized response. This prevents the need for a systemic antibody therapy, which would block proinflammatory cytokines that are in the RANK-L pathway (such as IL-1 [5]).

Specific Aims

The aims of this proposal are to explore the feasibility of releasing stable OPG therapies over extended periods of time, on a scale that is not typically considered for implanted immunotherapy approaches. The OPG stability should be retained for several years, something that has not yet been assessed in literature, and will require future testing considerations to emulate storage. The delivery of OPG doses will be dependent on the extent of cement degradation, which releases the therapy, so anti-inflammatory responses from a wide range of doses must be assessed. The questions raised above will be addressed through 3 specific aims:

1. *Assess the ability of encapsulated OPG to prevent osteoclast activation and maturation.*
2. *Form a stable microsphere for bone cement stability, but degradation when stressed.*
3. *For varying doses, ensure adverse anti-inflammatory responses are minimized in-vivo*

Significance

Implants placed in the body are becoming more commonplace, becoming routine procedures in the case of joint replacements in aging populations (See Figure 1). There are over 1 million titanium hip replacement surgeries performed annually in the US, with 10-20% requiring revision [1]. One of the several impacts from long-term implantation is the release of wear particles, or debris from the implanted material, at its points of articulation with the body. Wear particles are created through abrasion between implanted surfaces (such as during joint articulation, or incidental contact), micromotions between an implant and its anchoring interface (such as bone or cement), a surface coating applied to the implant, or even corrosion from passage of time [6]. These particles are mostly metal or polymers, which are common

materials in prosthetic joints, and can be nano- or micro-particles which are associated with inflammatory reactions, implant rejection, and osteolysis.

Research has been conducted on decreasing initial wear particle response by careful selection of materials, and probabilistic tuning the resulting particle sizes [8, 9]. Biological strategies against wear particles are now looking at combating immunological effects by interfering with macrophage uptake and trafficking, modulation of macrophage phenotypes, and local inhibition of RANK, as well its component nuclear factor-kappa B (NF-κB) [10]. A novel solution would be one that can act locally, on or near osteolysis surrounding the site of the implant and provide a targeted solution without requiring systemic therapy administration. Osteolysis, which is the degradation of bone, can occur where there is chronic activation of the immune system by responses against the implanted material which promote osteoclast activity. Osteoclasts are present and active throughout the skeletal system along with osteoblasts (which lay down new bone material), as together (See Figure 3) they replace about 10% of our skeletal system every year [11]. By preventing the progression of osteolysis and the activity of osteoclasts, time is gained for osteoblasts to fill in and replace bone that has been degraded away.

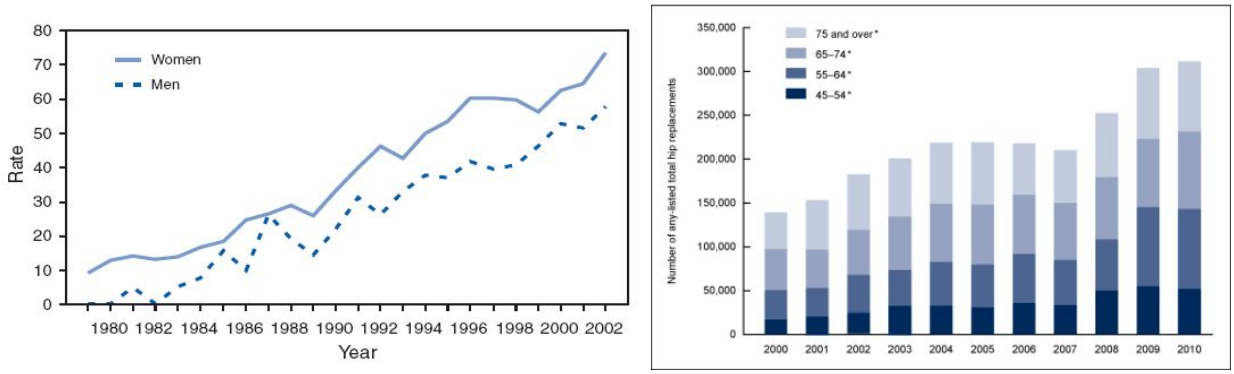

Figure 1 - Occurance of Implants and Revisions in US populations. *Left*: The Rate per 10,000 people of total knee replacements for those aged ≥ 65 or older between 1979-2002. The data from the CDC presents a 7-fold increase over 23 years in the incidence of total knee replacements *[F1-1]*. *Right*: US hip replacements among inpatients aged ≥ 45 and over by age group and year (2000–2010). This CDC data shows an increase in all age groups especially those 55-64, who are likely more active with their prosthetic joints and benefit from revision surgeries *[F1-2].*

A Case Study, and Patient Futures

As a case study, looking at total knee arthroplasties in 2006 [12], there were slightly under 60,000 revision procedures performed. Of those, 25% were due to infection, 16% due to loosening, 3% from periprosthetic osteolysis, and 5% due to surface wear. From the sampled data, the revision procedure with hospitalization on average lasted 5.1 days, and cost $49,360 to perform [12]. Patients lives can be improved by extending the duration from implantation until revision is needed. In order to reduce patient discomfort leading up to revision, reduce healthcare costs associated with increasing surgeries, and prevent surgical pain to patients -- the duration of time between implantation and revision can be targeted, ultimately number of

revision surgeries needed. Reducing the rate of infection may be explored in future studies by co-delivering therapies in the embedded microspheres as well.

Innovation

This immunoengineering project seeks to deliver a targeted approach for osteoclast damage, as opposed to the systematic approaches that have been previously explored. This will be accomplished via OPG release hyperlocally at the site of bone damage, which will act on nearby osteoclasts and osteoclast precursors. The OPG will be encapsulated into microspheres are able to survive for extended periods of time within the body (targeting several years) before releasing being released into the body [13, 14]. The molecules will be released as the cement compound which interfaces between the prosthetic and the implant is worn away by osteoclasts or microdamage. Osteoclasts are differentiated and matured in response to the release of RANK-L by macrophages, activated by wear particles (See Figure 2).

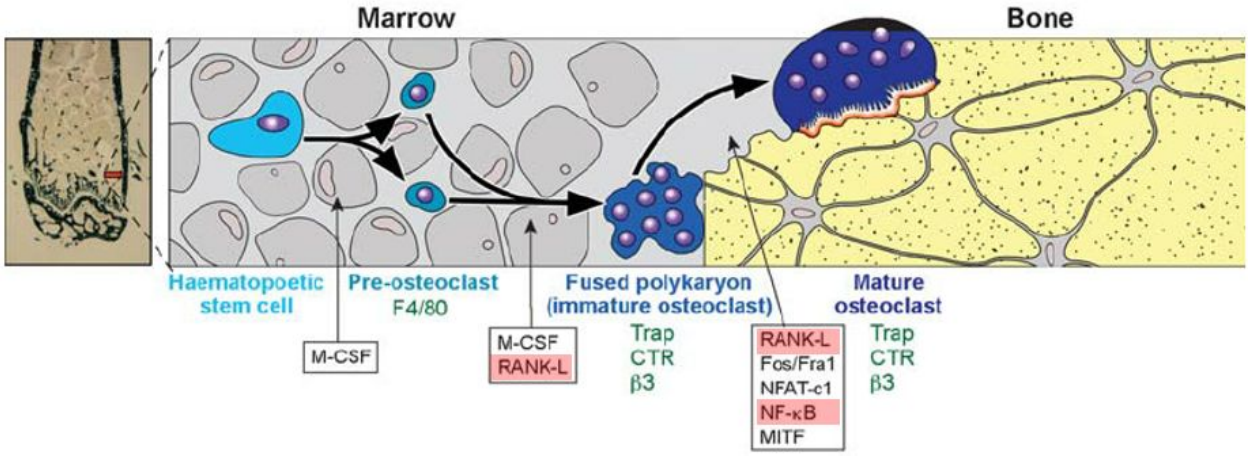

Figure 2 - Regulation of osteoclasts from bone marrow to bone osteolysis. Osteoclast precursors (pre-osteoclasts) require regulation in two phases in order to become activated (quiescent or immature osteoclasts), and then mature osteoclasts. In both steps RANK-L is a regulator; if not consistently provided to mature osteoclasts they will revert to their immature state, and is required for osteoclast activation. NF-κB, also highlighted in red, may also be encapsulated as it is a component of RANK-L *[F2]*.

Challenges to current approaches are that they tend to focus on the material properties, inert surface, surface biofilms, or other coating, of the implant, or on systemic administration of immunosuppressant drugs. The novelty here is that the therapy and treatment will be applied through the bone cement that is used to interface the implant with the body, allowing for a localized drug release at the site of the cement. The concept of embedding microspheres into construction (not bone) cement is not a novel concept, as self-healing polymers and fungal particles have been used in the past [15], but for bone cement this appears to be limited to the release of antibiotics targeting release only over several weeks [16, 17].

The ability to store immunotherapies for long periods of time upto and including several years, and then and then release the contents of microspheres would be a benefit to the scientific

field. This could be extended not only for OPG, but to anti-inflammatory cytokines, pro-osteoblast regulators, and treatments to fight infections, which as mentioned are responsible for 25% of knee arthroplasty revisions.

Approach

During the course of evaluating the local and systemic response to the encapsulated microspheres, the preliminary studies will be bench-top assessments of cements and the embedded microsphere endurance to satisfy Aim 2. Studies of microspheres mixed into the cement can be checked for consistent distribution (no microsphere settling), exothermic temperature curves, curing rates, hardness, as well as crack patterns. The stability of microspheres over simulated multi-year time courses is a challenge that will need to be investigated further. Next, the interaction of the microsphere doped cement with bone and implants in in-vitro (or laboratory created conditions) will be assessed - utilizing the same bench-top benchmarks. The in-vitro conditions will be helpful in identifying potential issues that would be encountered in-vivo such as setting of the cement against damage bone or surrounding tissue. One factor that will need to be carefully studied is the abrasion of microspheres against the coating of the implant, which may be a biofilm, or carefully prepared surface designed for enhanced osseointegration. The final material of these microspheres will need to be chosen such that it provides a stable environment for the OPG while embedded in the cement, but is also able to rapidly rupture and release OPG after it is compromised by osteoclasts or microdamage resulting from bone degradation.

Microsphere exposure and OPG release will be tested in-vitro through simulated osteolysis performed by chemically degrading the bone and cement while monitoring for release over time. Imaging and histology on bone, tissue, and cement samples will ensure that the microspheres are not impacting the bone-cement-prosthetic adhesion required for prosthetic stability, but also are able to survive unruptured inside the cement, providing a stable environment for the OPG protein until it needs to be released.

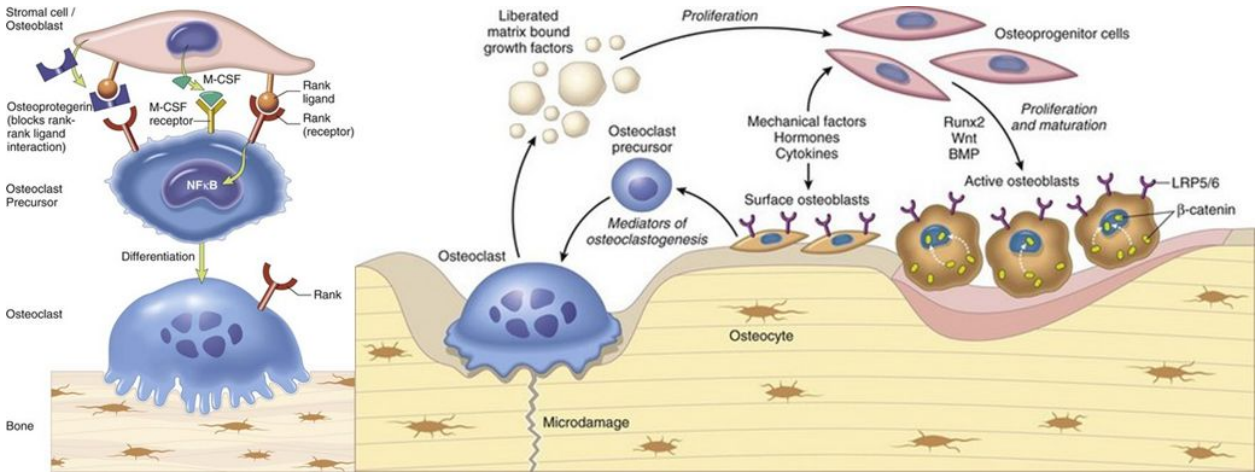

Figure 3 - Osteoclast inhibition by Osteoprotegerin, and osteoblast/osteoclast activity in bone. *Left, Top:* The activation (differentiation) of osteoclast precursors into of osteoclasts is mediated by RANK/RANK-L and M-CSF/M-CSF-R. Osteoprotegerin (OPG) blocks the RANK/RANK-L interaction, and prevents the release of NF-κB as well as the activation of osteoclasts). *Left, Bottom:* The activated osteoclasts, are also called immature osteoclasts, and require further interaction of RANK/RANK-L as

seen by the RANK receptors still present on the cell surface. OPG inhibits blocks this interaction as well, preventing the maturation of osteoclasts and the degradation of bone. *Right*: The role of osteoclasts and osteoblasts in the degradation, and growth, respectively, of bone. Source: Modified from *[F3]*.
In vivo conditions would require an assumption that not all of the OPG protein would maintain its stability over time, so microspheres will likely contain large amounts of OPG, such that microspheres released initially release excess OPG, causing small immune responses, but microspheres still released months or years later contain sufficient viable OPG to block RANK/RANK-L interactions (see Figure 3).

Both Aims 1 and 3 require experimentation with the dose quantities of OPG releases for intended (Aim 1) and unintended (Aim 3) effects. For the intended effects in Aim 1, an in-vitro simulated environment will be created where osteoblasts can degrade bone and be administered varying doses of OPG to assess their effects, varying from overdosing (initial release) until there is no statistical change in activity, at which point there is no longer sufficient stable OPG in the microspheres to produce a functional effect. In order to accurately test the localized responses, microspheres with varying concentrations of OPG can be released into the immediate vicinity of the immature and mature osteoclasts, and their activity can be monitored via imaging and histology. Following this, microspheres of the final material as chosen in Aim 2 can be fabricated with varying quantities of OPG and embedded in cement for in-vivo validation using animal models.

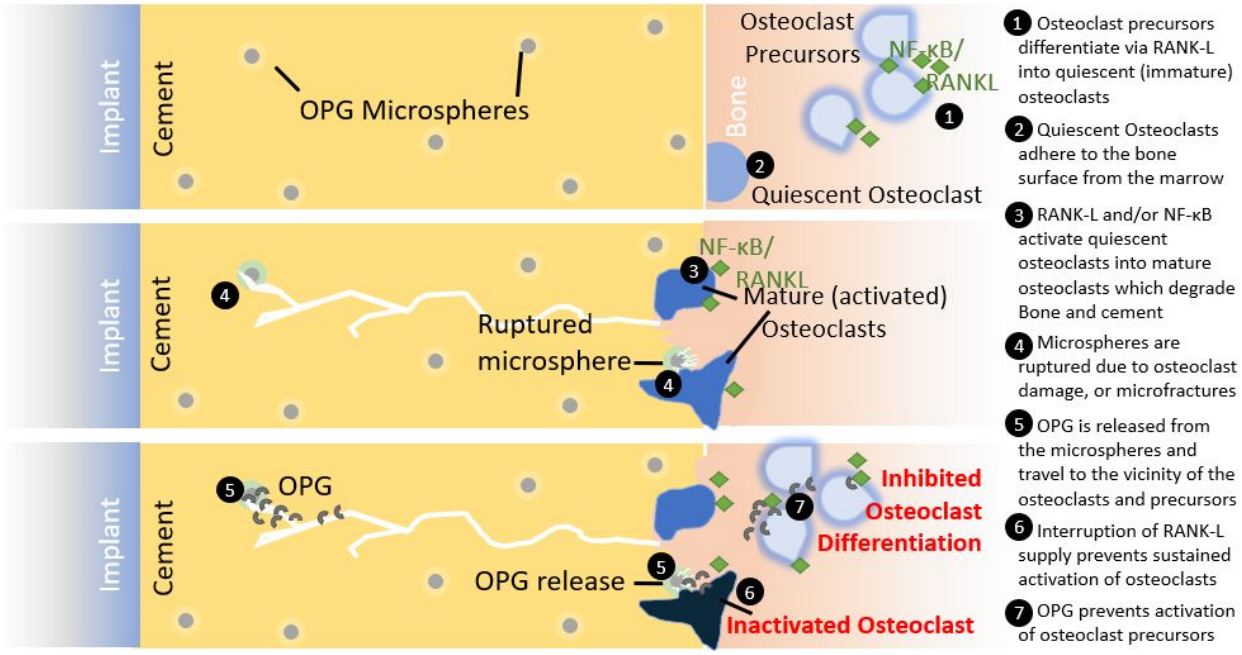

Figure 4 - Overview of the proposed OPG filled microspheres for localized blocking osteoclast activity. The proposed solution involves blocking the RANK/RANK-L interactions on the surface of osteoclast precursors (1) and quiescent (or immature) osteoclasts (2). These are activated by RANK in response to immune activation, in this case from wear particles being released from a joint implant. As osteoclasts degrade the surface (3) of the bone and the nearby bone cement, embedded microspheres in the

cement are ruptured (4) and release their encapsulated OPG protein, which blocks RANK/RANK-L interactions (5). The removal of RANK-L stimulation reverts activated mature osteoclasts into immature osteoclasts (6), and prevents further osteoclast precursors from differentiating (7). *Source: Self.*
Aim 3, which seeks to minimize excessive anti-inflammatory responses, will be performed using in-vivo validation of the doses decided on based on effective OPG blocking of RANK/RANK-L interactions. One consideration that must be taken into account here is that if the prosthetic requires revisions before the functional end-of-life of the OPG and microspheres, the surgical removal of the prosthetic and the drilling of the cement will release a bulk-release of OPG into the bone marrow and surrounding tissue.

The theoretical quantity of OPG released during revision by the microspheres can be discovered by proxy by filling the microspheres with an marker material that will not be taken up by the body, or bind to surrounding surfaces and perform a revision using an animal model. The resulting bone, tissue, and medical waste from drilling should be captured, and the marker extracted, to assess the quantity released. This volume should be accounted for in future surgical revisions.

Issues and Difficulties
Once microspheres have been ruptured and the OPG released, they are no longer able to provide an effective therapy. If osteoblasts were able to rebuild bone over the exposed microsphere, it is possible that the time until revision or failure of the prosthetic implant is minimized. The continued release of wear particles will drive bone degradation, and it is not until microspheres embedded deeper into the cement are encountered and OPG is released that the next wave of RANK/RANK-L blocking will occur. Because the initial portions of this research can be performed using bench-top and in-vitro experimentation progress can be made prior to IRB and FDA considerations. As multi-year stability and in-vivo models are required the development of this technology will slow down, impacting its applicability for commercialization.

Conclusions
In summary, the number of joint prosthetics that are implanted in the US are increasing year after year, and are being increased into younger patients as well. As a result of joint implants wear particles are released that cause an immune response leading to osteolysis or breakdown of bone. In addition to ongoing research and improvements for prosthetic devices and systemic immunotherapies. I am proposing a bone cement based solution that can be utilized along side current and future joints/immunotherapies. The innovation is in stable microspheres embedded into cement release therapy (the protein OPG) over several months/years, instead of days/weeks as is currently done with antibiotic therapies embedded in bone cement. OPG has been well studied for its powerful suppression of osteoclast activity responsible for osteolysis. As the bone and neighboring bone cement is broken down by osteoclasts, the microspheres embedded within the bone cement will rupture releasing OPG which blocks RANK/RANK-L interactions, inactivating osteoclasts, and preventing further differentiation from osteoclast precursors. The impact of this is allowing osteoblasts to rebuild the degraded skeletal and cement structure strengthening the osseointegration and prolonging the duration until implant failure or revision is necessary. This will prevent pain, delay the amount of time

required until revision surgery is needed (if at all), and reduce the lifespan of the implanted prosthetic joint.

References: Paper Text then Figures *(prefixed with Figure number then reference: F1-2)*

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[F1-1]
[F1-2] Wolford, M. L., Palso, K., & Bercovitz, A. (2000). Hospitalization for total hip replacement among inpatients aged 45 and over: United States. NCHS Data Brief, 2010(2015), 1-8.
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