ORTHOPAEDIC IMPLANT FAILURE Aseptic implant loosening: contributions and future challenges of mouse models in translational research

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Aseptic implant loosening: contributions and future challenges of mouse models in translational research

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Abstract

Aseptic loosening induced by UHMWPE wear particles is considered the main cause of long-term implant failure in orthopaedic surgery. The current therapy consists of surgical revision and implant replacement, carrying severe consequences for public health. Over the past 20 years, preclinical approaches including mouse models have been essential in the advances made in the understanding of the pathophysiology of periprosthetic osteolysis involved in the loosening process. However, while encouraging results have been obtained *in vivo*, there is still no valid clinical alternative to revision surgery. This review provides an overview of the current approaches used to study the biology of periprosthetic osteolysis and discusses the contributions, limitations and future challenges of mouse models for a successful translation of the preclinical advances into clinical applications.

INTRODUCTION

Total hip arthroplasty (THA) is one of the most successful procedures performed today and its indication in the younger and active population is increasing [1]. Among the bearing surfaces available, metal-on-ultra-high-molecular-weight-polyethylene (UHMWPE) has been the most indicated [2]. However, up to 20% of patients show evidence of aseptic osteolysis within 10 years [3], leading to implant loosening and surgical revision [4]. Projections for the year 2030 estimate that the indication of THA and surgical revision will increase to 154% and 137%, respectively, carrying severe economic consequences in public health [5]. Thus, long-term implant survival continues to be a major challenge in orthopaedic surgery. In this regard, a better understanding of the pathophysiology of UHMWPE-induced osteolysis is essential for therapeutic development.

Among the approaches which have been proposed to study the biology of aseptic osteolysis, only *in vivo* assays allow the examination of the overall pathophysiology of osteolysis. While different animal species have been used for this aim [6,7], mouse models have become one of the most relevant tools to improve the current knowledge in this field, highlighting the pathophysiology and proposing encouraging research strategies [8,9]. On the other hand, clinical studies have shown the retrospective data of the disease; however no long-term preventive protocol has been established to date [10].

Considering the important preclinical progress made in the past decade, some relevant questions can be raised, such as: why has this progress so far not been translated into clinical success? Are the current models profitable and pertinent? What are the future challenges for a better understanding of the pathophysiology of aseptic osteolysis? The present review gives an overview of scientific approaches for the study of particle-induced aseptic loosening and more specifically highlights the contributions and future challenges of mouse models. Finally, the difficulties in transferring the knowledge from preclinical approaches into the clinic are discussed.

TRIBOLOGY OF ORTHOPEDIC BEARING SURFACES

The tribology of artificial joints comprises the wear behaviour of bearing surfaces of implants [11]. During the wear process, worn sub-micrometric particle debris is released normally or abnormally from the bearing surfaces to the bone microenvironment [11]. The wear particles cause secretion of pro-inflammatory cytokines by macrophages, production of pro-resorptive cytokines by osteoblasts and fibroblasts, stimulation of osteoclastogenesis, induction of osteolysis and subsequently the loosening of the implant (Fig. 1). The sources of particles can be classified into metallic and polymeric, the former including titanium (Ti) and chrome-cobalt (Cr-Co) and the latter consisting of UHMWPE from acetabular cups and polymethylmethacrylate (PMMA) from cement. While wear debris of Ti, Cr-Co or PMMA is released under abnormal function of the prosthesis, UHMWPE particles are released normally during the life-span of the prosthetic device [12]. Thus, production and accumulation of UHMWPE wear debris is an inherent phenomenon of implant use [12].

The use of metal-on-UHMWPE bearing surfaces in hip implants was proposed by Charnley in 1961. This low frictional system is comprised of an acetabular cup of UHMWPE articulating against a small diameter metallic femoral head [13] (Fig. 2). Its success is due to the combination of excellent mechanical properties and biocompatibility; however UHMWPE, which has become the most commonly used material as a prosthetic surface after the failure of polytetrafluoroethylene (PTFE, Teflon) [2], has shown limitations in the long term due to the generation of debris [14]. In the past decade, a first-generation cross-linked polyethylene has shown lower wear rates and a decreased incidence of periprosthetic osteolysis compared to UHMWPE in the medium term; however the problem seems to persist: The cross-linked polyethylene still releases wear particles of a phagocytable size,

which is strongly related to its higher bioactivity [15]. Furthermore, the residual oxidation and potential for material fracture raise questions about its effectiveness. Recently, a second-generation cross-linked polyethylene, the highly cross-linked polyethylene, has been developed. New methods of stabilization (annealing and remelting) and incorporation of vitamin E (blended polyethylenes) lead to reduced oxidation and fracture risk and a lower rate of wear [14]. A recent long-term multicentric study examining the benefits of highly cross-linked polyethylene showed an improvement in the prognosis of implant survival in patients after THA of up to 13 years [16]. However, despite these advances, the role of wear particles in aseptic loosening has been the most relevant subject of research in the mechanistic description of periprosthetic osteolysis in the past 20 years. UHMWPE will remain the biomaterial of reference for the foreseeable future in order to assess the biological effects of new polyethylenes.

Other currently used alternatives for total hip arthroplasty include ceramic-on-ceramic and metal-on-metal bearing surfaces. These have shown better mechanical properties and lower wear rates than the classic metal-on-UHMWPE couple and represent a potential alternative to reducing the prevalent osteolysis and aseptic loosening in THA [17]. However, the persistent risk of fracture for ceramic [11], the potential risk of development of hypersensivity reactions [18] and chronic exposure to metallic ions with metal-on-metal couples [19] cast doubt on the real advantages over metal-on-UHMWPE couples. Finally, although modular systems have shown advantages in adapting to anatomical difficulties, there is a risk of micro-displacement of metal interconnectors and wear production [20].

APPROACHES FOR THE STUDY OF UHMWPE PARTICLE-INDUCED OSTEOLYSIS

Clinical trials and tissue retrieval

Clinical reports recognize osteolysis induced by UHMWPE wear debris as the main cause of aseptic failure leading to revision surgery [21]. Controversial results from preventive pharmacological therapies have been reported. While preclinical studies have shown that targeting some of the main cytokines involved in bone remodelling such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-10 and osteoprotegerin (OPG) results in a significant inhibition of bone loss around implants, suggesting a good clinical response, clinical trials failed to confirm this hypothesis in the long term [10].

Furthermore, the inter-individual variability in implant survival after THA has been associated with the amount and/or activity of several cytokines from the IL-1 family as well as IL-6, based on their gene polymorphism, suggesting a potential genetic predisposition for the development of osteolysis [22]. These findings could facilitate the pre-operative identification of patients at risk of developing periprosthetic osteolysis and implant failure. In brief, despite the symptomatic aspects and a potential genetic predisposition for implant loosening, there remains a lack of understanding of the natural history of the disease. However, the analysis of periprosthetic membranes removed during revision surgery has led to the characterization of the particulate and cellular infiltrate once the disease is installed [23]. Histological analysis has documented a fibrous granulomatous reaction, consistent with a foreign-body inflammatory response [12]. Many studies have confirmed the predominance of wear debris of UHMWPE in periprosthetic tissues [24,25].

Cellular profiling highlights the role of macrophages in this inflammatory response. Interestingly, a polarization from a pro-inflammatory subpopulation (M1) to an anti-inflammatory phenotype (M2) has been reported [26]. Cytokine profiling confirmed an elevated expression of IL-1, IL-6 and TNF-α in human pseudomembranes [23]. Furthermore,

the presence of matrix metalloproteinases (MMPs) (MMP-1, MMP-2, MMP-3 and MMP-9) has also been reported [27].

Globally, the efficacy of new drugs or optimized biomaterials needs to be confirmed in further randomized studies including longer follow-ups and relevant outcomes [10].

In vitro studies

In vitro approaches have served to highlight the interactions between particles and the most competent cells involved in periprosthetic osteolysis: macrophages, osteoclasts and osteoblasts. However, while most studies using established cell culture systems have been carried out with PMMA, Ti or Cr-Co particles [28], very few have used UHWMPE due to technical problems in generating particles that are clinically relevant. Furthermore, the lower density of UHWMPE particles leads to their separation from the cells (e.g. macrophages) attached to the bottom of a cell culture plate, preventing their activation [29]. However, Ingram *et al.* and Fang *et al.* have proposed two new methods to overcome these technical difficulties: the use of agarose gel and the inverted cell culture system [30, 31]. Both methods ensure physical contact between UHWMPE particles and macrophages.

Macrophages and wear particles. There is strong evidence that points to macrophages as the key cells implicated in periprosthetic inflammation and osteolysis by UHMWPE wear debris [12]. The presence of wear particles within phagocytable range (0.1 – 10 µm) inside macrophages suggests that the cells actively engulf particles, which subsequently leads to the release of pro-inflammatory mediators such as TNF-α, IL-1β, prostaglandin E2 (PGE2), granulocyte macrophage colony stimulating factor (GM-CSF) and IL-6 into the cell culture supernatant [12]. The expression of inflammatory cytokines in macrophage-particle cultures can be modulated by the particle composition [4], size [32], shape [33] and surface area [34]. However, it remains unclear which parameters are the most important [33]. Regarding the size, Endres *et al*. and Pal *et al*. tested the effect of UHMWPE nanoparticles on monocyte/macrophage response reporting an inverse relationship between particle size and bioactivity [35]. Activated macrophages were shown to induce a direct (by signalling of pro-inflammatory cytokines) or indirect (by increasing the osteoblastic-released RANKL) activation of osteoclastogenesis and subsequent osteolysis.

The receptors and intra-cellular mechanisms involved in the activation of the target cells are still poorly understood. Toll-like receptor (TLR) and Nod-like receptor (NLR) are two major forms of innate immune sensors that provide immediate responses against invasion or tissue injury [36]. Activation of these sensors by particles may therefore induce the recruitment of the cellular infiltrate, initiating an acute inflammatory process [36]. Moreover, the expression of estrogenic receptors (ERs) by periprosthetic macrophages has been recently reported and it has been shown that their blockade results in a decrease of TNF-α in murine macrophages activated by UHMWPE particles. The response to wear particles may therefore be modulated through ERs and their ligands [37]. With respect to the mechanistic understanding, Caicedo *et al.* proposed the NACHT, LRR, and PYD domains-containing protein-3 (NALP3) inflammasome, a macrophagic intra-cellular stress response triggered by the presence of phagocytized metallic debris [38]. Confirming this finding, the titanium-induced inflammation triggered by macrophage TLR signalling and intra-cellular activation of the NALP3 inflammasome has been shown to induce increased IL-1β secretion and IL-1-associated signalling and to promote the recruitment of neutrophils [39]. These events may therefore maintain chronic inflammation, induce periprosthetic bone loss and ultimately lead to implant failure [39]. In the same vein, a recent report indicated that PMMA, metallic particles and ions activate the NALP3 inflammasome and caspase-1 system in *in vitro* and *in vivo* models, proposing the inflammasome as a critical mediator of orthopaedic wear-induced osteolysis and as a viable therapeutic target for the treatment of periprosthetic osteolysis [40].

Osteoblasts-osteoclasts and wear particles. Normal bone formation by osteoblasts is essential for implant survival. Disruption of osteoblast function by wear particles was hypothesized to be an important factor influencing the balance in bone remodelling and thereby decreasing the survival of implants due to aseptic loosening. Several authors have reported an inhibitory effect of Ti particles on osteoblast function by various mechanisms: suppression of gene expression of collagen type 1 [41], apoptosis triggering [41] and alterations of adhesion [42] with a consecutive diminution of cell viability [41,43]. Moreover, different particle types and surfaces were tested, identifying cobalt-based and rough-surface particles as the most cytotoxic compositions and, inversely, zirconium oxide (ZrO_2) particles as the most innocuous [41,43]. Exposing osteoblasts to UHMWPE particles *in vitro* decreases their osteogenic activity (mineralization, proliferation, alkaline phosphatase activity, and osteocalcin production) mediated by an increase in receptor activator of nuclear factor κB ligand (RANKL) expression and induction of a catabolic phenotype [15,35].

The presence of UHMWPE wear particles in the periprosthetic bone microenvironment enhances the differentiation of osteoclast progenitors into mature osteoclasts in response to the cytokines released by activated macrophages and osteoblasts [44]. In the past decade, major advances in understanding the osteoblastic regulation of osteoclastogenesis, mediated by the RANKL/RANK/OPG system, have been made [45]. For example, it was shown that OPG expression by human osteoblasts was significantly inhibited in the presence of UHMWPE particles, leading to a decreased OPG/RANKL ratio and subsequent stimulation of osteoclastogenesis *in vitro* [48]. Furthermore, the intracellular activation of osteoblastic cells by phagocytosis mediator proteins ERK1/2-CEBPβ may be a key inflammatory pathway that links phagocytosis of wear particles to inflammatory gene expression in osteoprogenitors cells [49].

In vivo studies using mouse models

Different species have been used to test biological and biomechanical parameters, two aspects closely related with aseptic implant loosening [50]. These include, sheep [6] dogs [51], rabbits [7], rats [50] and mice [9,53]. The use of large animals has obvious advantages, especially if the desired outcome is the global understanding of the behaviour of the implant in the host. However, the cost of maintenance and the necessity of multiple therapeutic interventions in a large animal do not make these options the most used as a first *in vivo* approach. For this reason, murine models appear to be the most effective alternative, due to their low cost of maintenance, the facility to reach sufficient numbers of individuals to strengthen statistical results and the diverse options in genetic or immune features available [9,54]. Mouse models have been exploited with two major aims: the reproduction of a human clinical phenotype (modelling) and the development of therapeutic assays.

To model particle-induced osteolysis *in vivo*, it is necessary to establish a causal effect between the particles and inflammatory osteolysis and to unveil the mechanisms involved, identifying their critical markers. The first aim has been well achieved in mice, demonstrating that the UHWMPE-induced formation of granulomas and subsequent development of osteolysis is highly dependent on the size and shape of the particles [12]. Moreover, Wooley *et al.* suggested an important role of UHMWPE particle size, introducing the concept of a "critical particle size" (<20 μm) required to induce significant reactions *in vivo* [53]. The desired second aim of unveiling the mechanism of particle-induced osteolysis is based on a classical paradigm: macrophage-mediated inflammatory responses and increased osteoclastogenesis lead to an imbalance between bone formation and resorption [48]. In this sense, in the past decade the biological interactions between particulate debris and immune response have been better understood. Goodman suggests a differential immune response according to the biomaterial involved [18]. While polymers (UHMWPE and PMMA) seem to

trigger an unspecific response, metals (Cr-Co and Ti) are thought to trigger a type IV hypersensitivity reaction. The cellular effectors in the non-specific (polymeric) response appear to be mainly macrophages and fibroblasts, with a secondary modulator role of lymphocytes. By contrast, during the specific response T lymphocytes may play a major role in the maintenance of chronic inflammation, however their exact role in implant-loosening is still unknown [18]. Consequently, understanding the exact role of macrophages and lymphocytes is key to understanding the immune modulation induced by particles.

Several animal models have demonstrated an activation of macrophages in response to a variety of wear debris particles. Goodman *et al.* reported a foreign body giant cell reaction and prosthetic loosening that generated elevated levels of PGE2 in rabbits [54]. Spector *et al.* showed that macrophages produced elevated levels of PGE2 and IL-1 in a canine osteolytic model [55]. These findings were also confirmed in rat models [56]. Furthermore, new advances in *in vivo* imaging have served to confirm macrophage trafficking and have revealed the role of monocyte chemoattractant protein-1 (MCP-1) in macrophage recruitment in the presence of UHMWPE wear particles *in vivo* [57], as well as the role of transcription factor NF-κB in UHMWPE particle-induced osteolysis [58].

Interestingly, a paradoxical role of estrogens in mice has been recently proposed. While a classical bone protective function has been attributed to estrogens in humans, a contrary effect has been shown in mice, where the deprivation of estrogens was associated to a protective effect in a particle-induced osteolysis model [37]. These findings support a pro-inflammatory and pro-osteolytic effect of estrogens in periprosthetic tissues in mice, probably mediated by estrogen receptors expressed by periprosthetic macrophages [37,59]. Furthermore, an immunomodulatory effect of IL-4 on periprosthetic macrophages activated by UHMWPE particles has also been observed. IL-4 produced by activated macrophages seems to exert immunomodulatory activity through the polarization of macrophages from the pro-inflammatory sub-population (M1) to the anti-inflammatory M2 phenotype [26,37].

The development of immunologically compromised mouse models has served to sustain the concept of the involvement of the immune response in particle-induced osteolysis [38,39,60]. Lymphocytes and macrophages are considered the main cellular targets in this process. Taki *et al.* reported a similar osteolytic response to polyethylene or Ti particles in a double knock-out (K.O.) calvaria model of lymphocyte-deficient mice [61]. These results suggest that lymphocytes are not implicated in the osteolytic mechanism. Consistent with these findings, Purdue *et al.* reported a similar capacity to develop granuloma and osteolysis under polymeric stimulus in lymphocyte-deficient mice [4]. However, a lymphocytic infiltrate has been observed around metal-on-metal arthroplasties and this is correlated with a poor clinical implant performance [4]. Taken together, these results suggest that lymphocytes may be implicated in a metal-specific response, but not in a polymeric-nonspecific-response. Finally, Ren *et al.* highlighted the key role of macrophages in the UHWMPE particle-response in nude mice, showing the systemic trafficking of reporter macrophages and localized osteolysis after polymeric particle stimulation [62]. Despite these important advances, further studies in osteoimmunology are necessary to clarify their real importance.

Local osteoclastic bone resorption is influenced by different cytokines in *in vivo* models. Among these, TNF-α seems to play a main role since it stimulates directly osteoclast formation, differentiation and activity [44]. However, the essential cytokine network proteins controlling the recruitment of functional osteoclasts in wear particle-induced osteolysis *in vivo* seem to be the proteins of the RANK/RANKL system [63]. UHMWPE particles induce inflammation, but not osteoclastic bone resorption in $RANK^{-/-}$ mice [63]. However, other routes of osteolysis activation have been reported. Yao et *al.* and Nakashima *et al.* unveiled the capacity of macrophages and fibroblasts activated by wear particles to initiate directly the

resorption mediated by MMP-1, MMP-2, MMP-9 and osteolytic enzymes (e.g. collagenase and stromelysine) [64,65].

The identification of relevant inflammatory and osteolytic markers in mice includes biomarkers that can be found in clinical and *in vitro* studies as well. Among those are the cells of the immune response, osteoprogenitor cells, pro-inflammatory (IL-1, IL-6, TNF-α, PGE2 and others) and pro-osteoclastic cytokines (RANK, RANKL, IL-1 and TNF-α), pro-osteolytic factors such as MMP-2 and MMP-9 [66] and neuropeptides (calcitonine and α-CGRP) [67] or vascular signals (vascular endothelial growth factor [VEGF]) [68].

Current mouse models

The air pouch model. Wooley *et al.* developed a model to study the cellular reaction and expression of cytokines after the introduction of metals (Co-Cr, Ti-6Al-4V) and polymeric biomaterials (UHMWPE and PMMA) within subcutaneous spaces previously created, named "air pouches"[69]. Ren *et al.* modified this model by co-implanting bone sections from syngeneic mice and particles into the air pouches [51] (Fig. 3A). These studies showed macrophage infiltration and production of pro-inflammatory cytokines in the bone inside the air pouch, which then undergoes resorption [51].

The air pouch model has served as an initial "prove of concept" in the targeting of anti-inflammatory or anti-resorptive events related to particle-induced osteolysis. However, this model fails with respect to the reproducibility of the normal anatomic condition, because the bone is implanted in an ectopic site without its usual physiological environment, including blood supply, vascular, nervous and hormone control. These factors could be key aspects in the development of particle-induced osteolysis, considering the role of vascular and nervous peptides and soluble factors (hormones, cytokines and growth factors) involved in the control of bone homeostasis.

The calvaria model. In the calvaria model, a 1×1-cm area of calvaria is exposed by a midline sagittal incision with careful conservation of the periosteum, and particles are then placed on this surface [70] (Fig. 3B). This model has served as an important tool to understand the mechanisms involved in inflammatory bone resorption in a physiological bone site [71]. Merkel *et al.* showed that Ti particles lead to inflammation, osteoclast formation and bone resorption and proposed a key role for TNF-α in periprosthetic osteolysis after blocking the action of TNF-α by deletion of genes encoding its receptors (p55TNF and p75TNF) [70,71]. Other mediators such as IL-1 and IL-10, as well as cyclooxygenase (COX) 1, COX2, and PGE2 have been studied with this model. The injection of IL-1 stimulated bone resorption, demonstrating the responsiveness of the calvarial model to this inflammatory mediator [72]. The anti-inflammatory cytokine IL-10 has been shown to suppress wear debris-induced osteolysis [73]. Finally, COX2-deficient mice showed significantly less bone resorption induced by titanium particles than wild-type and $COX1^{-/-}$ mice [74].

In general, the main benefits of this model are the ability to test the host response in an orthotopic bone site, the possibility to split the calvarium and perform serial analysis, the rapidity of the development of osteolysis (about 7 days), the relatively low cost, the ability to screen a large number of experimental variables and the possibility to use small animal imaging to quantify bone loss. This model has permitted the use of transgenic strains in which the role of specific genes can be assessed for a better understanding of the pathophysiology and preclinical treatment of periprosthetic osteolysis. However, disadvantages are related to the fact that the calvarium consists of flat bones with membranous bone formation, rather than the endochondral ossification seen in long bones. Moreover, this model exhibits an acute (rather than chronic) effect and a lack of biomechanical factors related to osteolysis associated to wear debris, such as the presence of a load bearing implant, oscillatory fluid pressures and mechanical forces.

Intramedullary implant models. A third generation of models have been proposed by Warme (2004) and then modified and improved by Wooley *et al.* and Goodman *et al.* [9,52,75–77]. All of these models coincide in a) the ability to analyze endochondral long bone behaviour in experimental conditions, b) the presence of intra-medullary load-bearing implants and c) the possibility to assess long-term effects of particles in the periprosthetic bone microenvironment. These models can be analyzed according to the anatomical site involved (femur or tibia) and the frequency of particle implantation:

The proximal tibia hemiarthroplasty model. The hemiarthroplasty model proposed by Yang in 2007 consists of a stainless steel or titanium rod inserted intra-medullary in the proximal tibia epiphysis (Fig. 4Aa). Mice receive intra-operative and later monthly infusions of titanium particles. This model corresponds to long-term knee implant failure (24 weeks) and allows characterization of biomechanical aspects such as the position of the implant, migration of the intra-medullary pin (by micro-computed tomography) as well as stability and traction resistance (pullout test). Furthermore, pathological features can be studied, e.g. the effects of titanium particles on the expression of pro-inflammatory and osteoclastogenic cytokines (IL-1, TNF-α, IL-6 and RANKL) in periprosthetic tissue [52]. Moreover, Shi *et al.* [77] described a significant infiltration of macrophages in the periprosthetic tissue accompanied by an increase in osteoclasts and Howship's lacunae and a decrease in new bone formation using this model. Early results from these experiments are promising and have confirmed that particles around the implants induce a pro-inflammatory response in periprosthetic tissues, and that the pro-inflammatory cytokines IL-1 and TNF-α are involved [52].

The distal femoral implant model with single implantation of particles. Proposed by Warme *et al.*, this model consists of the inclusion of an intra-medullary metallic load implant in the distal femoral epiphysis associated with an intra operative single peri-implantation of particles [75] (Fig. 4Ab). Several authors describe the long-term effects (2, 10, 20 and 26 weeks) of a single dose of titanium [8,76] or UHMWPE particles [78]. Using this model, IL-1 has been suggested as a modulator in the formation of the periprosthetic membrane [8]. Furthermore, a single dose of UHMWPE particles showed a long term effect in up-regulating pro-inflammatory factors (IL-1, MCP-1, IL-6 and TNF-α) derived from mononuclear cells, suggesting that this mechanism could stabilize the chronic inflammatory state in periprosthetic tissues [78]. However, this conclusion would seem unlikely since inflammation and formation of the membrane are likely required steps leading up to resorption. Furthermore, the neutralization of IL-1 inhibits potently the particle-induced osteoclast differentiation [72].

The distal femoral implant with continuous intra-medullary infusion. Although distal femoral implants and tibia hemiarthroplasty with discontinuous or single implantation of particles have the advantage of being loaded implant models, they fail in providing a continuous release of particles of clinically relevant size, shape and volume. To remedy this, the group of Goodman proposed another model in 2008 [9]. Based on the *in vitro* and *ex vivo* validation of particle-release using an Alzet® pump [79,80], Ma *et al.* demonstrated that this model can ensure the constant presence of particles released from a subcutaneous osmotic pump in the femoral intra-medullary cavity, mimicking the human condition [9] (Fig. 4B). They successfully infused UHWMPE particles into the intra-medullary femoral cavity during 4 weeks. The model was characterized by micro-architectural and immunohistochemical analysis showing a reduction in bone volume. Ren *et al.* suggested that macrophages are systemically recruited from the circulation and migrate to the implanted medullar cavity within 2 to 3 weeks [62]. These cells would stimulate local osteolysis and up-regulate known bone remodeling markers. An interruption of this migration is proposed as a potential therapeutic approach [62].

Certainly, intra-medullary implanted murine models constitute a closer approach to the human situation than air pouch and calvaria models. However, the surgical manipulation of the epiphysis with the implant insertion in the carved channel could increase the risk of pathological fracture, giving a potential bias in the findings.

While multiple models were developed, only air pouch and calvaria models have been used regularly to perform therapeutic tests. Potential clinical applications are summarized in tables 1 and 2.

CONCLUSIONS

Over the past decade, significant advances have been achieved in the study of the pathophysiology and therapy of periprosthetic osteolysis. However, despite the preclinical progress made, new insights gained using mouse models have not yet been successfully translated to the clinic. We analyze this failure with a critical view on the performance of the models, the benefits gained from the emerging knowledge and the future challenges for mouse models in particle-induced osteolysis.

Mouse models in aseptic implant loosening/failure - a benefit-cost analysis. The use of mouse models for the study of the pathophysiology of periprosthetic osteolysis has produced vastly more benefits than costs. Benefits include the biological fidelity, their effectiveness, reproducibility and dynamism and finally the diversity of the genetic and molecular tools available. The biological fidelity of mouse models is based upon their resemblance with the periprosthetic environment observed in humans, sharing the same mechanisms of corporal development, bone tissue organization and physiological control of bone remodelling. Furthermore, this species has a reduced life cycle, giving the advantage of a rapid development of pathological features. The effectiveness and reproducibility of mouse models has been demonstrated repeatedly, predicting bone behaviour under different conditions (e.g. drug therapy or surgical procedures). Several therapeutic strategies have been employed successfully in preclinical studies. Furthermore, mouse models have proven to be dynamic, evolving from air pouches to intramedullary devices and proving useful for the assessment of the oldest as well as the latest generation of biomaterials (i.e. wear from cross-linked polyethylene). The availability of genetically modified or immunodeficient strains and the abundance of specific molecular tools have permitted the identification of genes, pathways, cells or tissues involved in the pathology, improving our understanding of the mechanisms of this disease.

Limitations for the use of mouse models include the bias of a partial reproduction of the real human condition. Periprosthetic osteolysis pathogenesis includes the biomechanical interaction between a prosthetic device and a host. Given that mice are small quadrupeds, mouse models cannot reproduce the "load bearing effect" of the implant, since there are no devices available that mimic a total joint replacement in mice. Other disadvantages of murine models are the small animal size and the low amount of cancellous bone, making their surgical manipulation difficult.

Relevance of mice in the modelling of particle-induced osteolysis. We believe that there is no one animal model useful for all stages of research in this field. Periprosthetic osteolysis is a multifactorial condition, with a close interplay between biomechanical and biological aspects. Mouse models are the first step in *in vivo* research, indispensable for confirming previous *in vitro* findings and for the screening of biological factors involved in a dynamic system; however, we believe that they do not have the necessary performance to translate their advances directly into clinical trials. While there is a consensus on some issues such as the multifactorial nature, the biologically induced phenomena, as well as the role of particles in

periprosthetic osteolysis, there are others which are still controversial, such as the genetic predisposition, immune and endocrinological regulation of periprosthetic bone loss, pharmacological considerations in drug delivery as well as the biomechanical aspects involved in a loaded endochondral bone. These heterogeneous topics need to be confirmed and tested in complementary larger animal models (e.g.: sheep or equine).

Achievements and future challenges. Emerging evidence from mouse models has contributed to the understanding of particle-induced osteolysis, changing from the old concept of a "cement-derived pathology" to a "multifactorial condition" that depends on the close interplay between wear particles (tribology) and host susceptibility, including the bone tissue. Concerning the tribological aspects, it is widely accepted that UHWMPE particles within a critical size range are one of the most bioactive materials. Improved polyethylenes for bearing surfaces (i.e. cross-linked and highly cross-linked polyethylene), alternative prosthetic devices like hip resurfacing and modular prosthetic systems have been proposed. Moreover, the local cytokine-mediated control of periprosthetic bone loss has been better understood using mouse models. The uncovering of a key role of osteoclastogenesis and osteoclast-mediated osteolysis has created a link between recent and general concepts of bone biology, such as the role of the RANK/RANKL/OPG axis or the pro-osteolytic effect of cytokines TNF-α or IL-1 or, inversely, the osteoprotective role of IL-10. Indeed, the cross-talk established between osteoclasts, macrophages and osteoblasts/stromal cells has progressively become a consensus in the field of periprosthetic osteolysis, leading to the hypothesis of a periprosthetic bone niche. In the same vein, a systemic control of periprosthetic bone response, especially by the endocrine system, has been proposed. The involvement of calcitonin or estrogenic control of periprosthetic bone is an emergent and interesting topic to explore.

A genetic susceptibility to periprosthetic bone loss could be suspected after reports showing that a polymorphism for the gene encoding the cytokine IL-6 is associated with local control of osteolysis. However, one of the most transcendent consensuses is the implication of macrophages in the triggering of an inflammatory response. Clearly, this aspect demands a further look into the immune response and how it is related to particle-induced osteolysis. A potential role of TLR and the activation of the intracellular inflammasome pathway by particles have been proposed recently. Another advance made in this field has been the induction of inflammatory and osteolytic changes in the intramedullary site in mice, approaching the aim of "endochondral bone loading".

Finally, therapeutic progress made in mice includes the possibility of systemic drug delivery, via gene transfer or intravenous administration.

Future challenges (Fig. 5) for murine models include improving the understanding of the biological response induced by nanometric particles derived from improved polyethylene, as well as the potential cytotoxic response to metallic particles. Furthermore, the endocrine regulation of the periprosthetic environment (by calcitonin, estrogens or others) needs to be clarified. The genetic susceptibility could have high value and open the path for the development of novel inter-individual prognostic markers, however in order to exploit this knowledge a human genetic profile needs to be first established and then mimicked in mice.

The role of the immune response in the periprosthetic tissues seems to be an extremely relevant chapter for future research, considering the strong evidence that supports the involvement of macrophages and probably lymphocytes. The understanding of the cellular and molecular mechanisms involved in recruitment, differentiation and activation of monocyte/macrophage cells seem to be a central axis of research in the immunomodulation of bone response. Furthermore, pharmacological aspects related to drug delivery will allow clarifying the equivalence of animal doses, frequency and route of administration by taking into account human requirements. In this regard, nanotechnology may be a potential useful

tool to apply in this field. Finally, the current models of loaded intramedullary implants developed in mice need to be tested under controlled, preventive or curative protocols, using the same markers established in the calvaria model. This aspect would give more validity to the results, considering the human-mouse homology of the intra-medullary site.

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Table 1. In vivo assays using air pouch mouse models with clinical perspectives

Author	Model	Therapeutic intervention	Strategy
Yang S, 2002 [81] Yang SY, 2004 [82]	Air pouches, UHMWPE	IL-1Ra, viral IL-10	Blockade of IL-1 by gene therapy to prevent or retard the inflammatory response
Yang S, 2002 [83]	Air pouches, UHMWPE	Viruses encoding human OPG gene (rAAV-hOPG)	Inhibition of osteoclastogenesis by gene transfer targeting OPG
Ren, 2006 [84]	Air pouches, UHMWPE	Erythromycin	Reduction of cytokine production and osteoclast differentiation
Ren, 2007/Ren, 2011 [68,85]	Air pouches, UHMWPE	VEGF antibody and a VEGF receptor II inhibitor	Inhibition of inflammatory and osteoclastogenic response by blockade of specific monocyte/macrophage receptor
Zhang, 2011 [86]	Air pouches, Ti	Locally delivered lentivirus-mediated VEGF miRNA	VEGF gene silencing
Wang, 2012 [87]	Air pouches, UHMWPE	Local administration of adenovirus expressing siRNA-targeting BMPR-IB	Downregulating osteoclastogenesis through the RANKL-OPG pathway
Chen, 2012 [88]	Air pouches, Ti	Tetracycline	MMP-9 inhibition and downregulation of RANK/RANKL
Dai, 2012 [89]	Air pouches, metal	VEGF antibody (Bevacizumab)	Inhibition of inflammatory responses and osteolysis
Chen, 2012 [27]	Air pouches, Ti	p38 MAPK inhibitor	Downregulation of osteoclastogenesis

Table 2. In vivo assays using calvaria mouse models and clinical perspectives

Author	Model	Therapeutic intervention	Strategy
Schwarz, 2000 [71]	Calvaria, Ti	Pentoxifylline (anti TNF-α) and alendronate.	TNF-α inhibition in activated macrophages and antiresorptive effect in mature osteoclasts
Childs, 2001 [90]	Calvaria, Ti	Etanercept	Osteoclast depletion via TNF-α blockade
Zhang, 2001 [74]	Calvaria, Ti	Celecoxib (Selective COX-2 inhibitor)	Blockade of cross talk with pro-inflammatory cytokines and Inhibition of prostaglandin production
Carmody, 2002 [73]	Calvaria, Ti	vIL-10 (AdvIL-10)	Antiinflammatory, inhibition of osteoclasdtogenesis and osteolysis via gene trasfer of IL-10
Childs, 2002 [91]	Calvaria, Ti	Recombinant RANK-Fc	Osteoclast depletion via RANK blockade (RANK-Fc)
Von Knoch, 2005 [92]	Calvaria, UHMWPE	Zoledronic acid (Zol) and Simvaststin	Antiresorptives via intra-cellular mevalonate pathway blockade in mature osteoclasts
Von Knoch, 2005 [93]	Calvaria, UHMWPE	Recombinant Fc-OPG	Osteoclastogenesis inhibition via exogenous OPG (OPG:Fc)
Zhang, 2007 [94]	Calvaria, PMMA/UHMWPE	Doxycycline (DOX)	Inhibition of osteoclastogenesis, pro apoptotic of mature osteoclasts and inhibition of MMP
Landgraeber, 2009 [95]	Calvaria, UHMWPE	Anti apoptotic	Macrophage and osteoblastic anti apoptosis
Kauther, 2011 [96]	Calvaria, UHMWPE	Calcitonin substitution	Inhibition of osteoclasts activity
Rao, 2012 [97]	Calvaria, UHMWPE	IL-4	Modulation of macrophage polarization (M1/M2)
Nich, 2013 [37]	Calvaria, UHMWPE	ERαKO or ER pan-antagonist	Macrophage response mediated by estrogen receptors (ER)
Kauther, 2013 [67]	Aged calvaria, UHMWPE	Neuropeptides such as Calcitonin/α-CGRP	RANKL-inhibition in aged cal-/cal- model
Yamanaka, 2013 [98]	Calvaria, PMMA	MAPK/JNK inhibitor and calcineurin/NFAT inhibitor cyclosporine-A	Blockade of JNK and NFAT pathways
Burton, 2013 [40]	Calvaria, PMMA	Caspase-1 deficient mice	Blockade of caspase-1 (effector of the NALP3 inflammasome)

FIGURE LEGENDS

Figure 1. Molecular mechanisms proposed in particle-induced osteolysis. (**A**) Macrophage and osteoblast activation by wear debris particles. (**B**) Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, PGE2 and GM-CSF) are secreted by macrophages producing an initial inflammatory response. Among them, IL-1β and TNF-α (asterisks) are further capable of directly stimulating osteoclastogenesis. In parallel, IL-1β and IL-6 activate the indirect (osteoblastic) way of osteoclastogenesis, mediated by RANKL. (**C**) Increased level of RANKL secretion by activated osteoblasts induces up-regulation of the RANKL/RANK system (+++) with inhibition of the osteoprotective couple RANKL/OPG (---) in presence of M-CSF. (**D**) The osteoclastogenesis pathway includes the differentiation of osteoclast precursors into activated osteoclasts and subsequent osteolysis in the resorption pit (Adapted from Purdue *et al*. 2006 [4] and optimized by Elsevier's Illustration Services).

Figure 2. Metal-on-UHWMPE hip implant (Charnley system). (**A**) The UHWMPE acetabular cup (red star) with PMMA cement (blue star) is located around the prosthetic femoral head (PFH) (courtesy of Prof. Gouin, CHU Nantes, France). (**B**) A retrieved UHWMPE acetabular cup (red star) with evident signs of wear and metallosis (courtesy of Prof. Rosset, CHU Tours, France). (**C**) Schematic representation of the main source of polymeric wear debris, including UHMWPE from acetabular cups (red star) and the cement of PMMA (blue stars). The synovial fluid is moving from the *pseudo* joint capsule to the bone-cement and stem-cement interfaces (red arrows). Osteolytic areas are located in acetabular and femoral sites (black arrows) (Adapted from Howie *et al*., 1993 [99]).

Figure 3. Air pouch and calvaria models. **(A)** Air pouches for the bone resorption model. Co-implantation of bone sections (black dotted line) from syngeneic mice and experimental particles (yellow circles) in the well established subcutaneous pouch in a receptor mouse. (**B**) Calvaria mouse model. Surgical incision of 1 cm length (a), exposure and incision of the pericranium at the middle line (b) and over-spread of experimental particles (yellow circles) (c).

Figure 4. Intra-medullary implant models. (**A**) The proximal tibia arthroplasty model and the distal femoral implant model. A metallic rod (MR) is inserted within the medullar cavity by proximal tibia (PT) epiphysis approach (a) or by distal femoral (DF) epiphysis approach **(b).** Peripheral osteolytic areas are highlighted (black arrows) (Adapted from Ma *et al*., 2008 [9]; Yang *et al*., 2007 [52]). (**B**) The distal femoral implant with continuous intra-medullary infusion. Long-term knee implant failure model characterized by continuous release of experimental particles by osmotic pump inserted in a subcutaneous pouch (orange) and connected by a tube (green) with the intra-medullary femoral rod (black). (Adapted from Ren *et al*., 2011 [62])

Figure 5. Translational challenges in orthopaedic aseptic loosening.

FIGURES

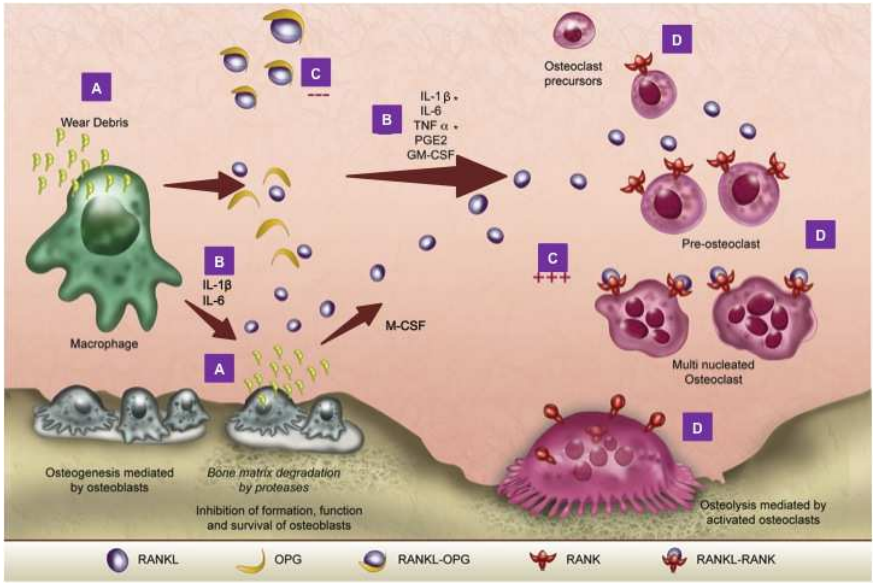

Figure 1

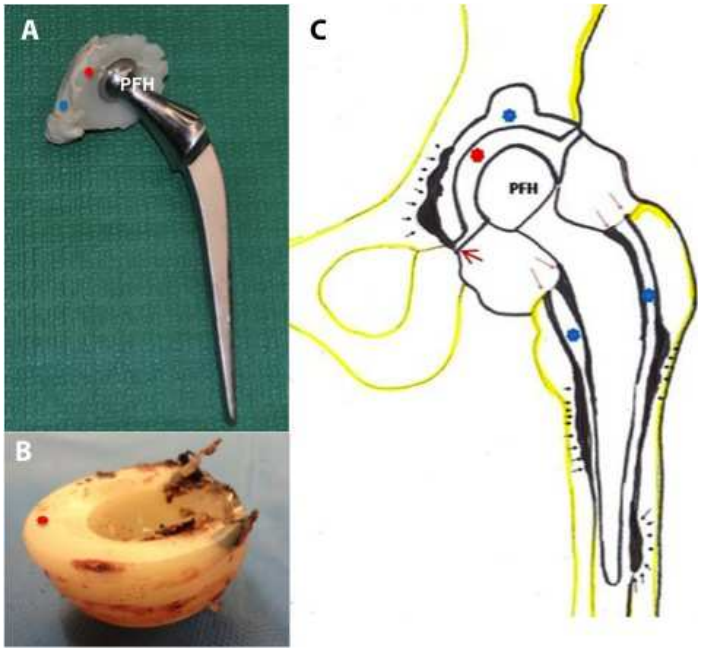

Figure 2

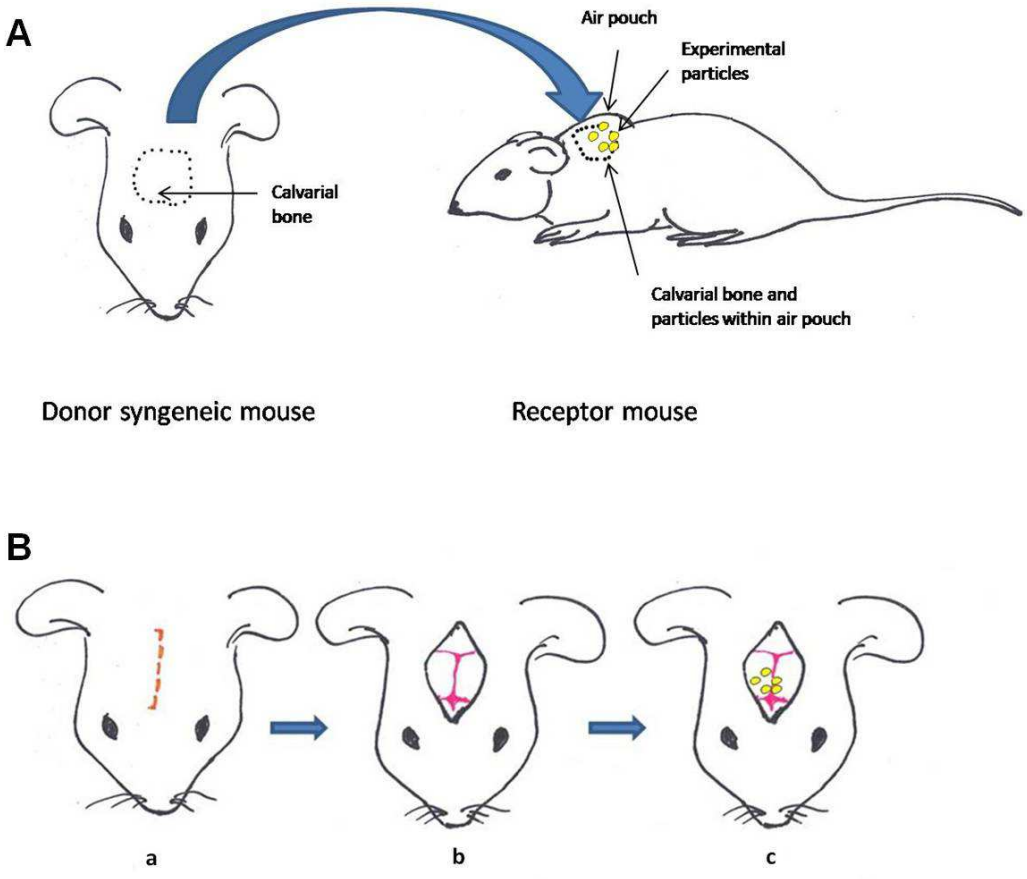
A
Air pouch
Experimental particles
Calvarial bone
Calvarial bone and particles within air pouch
Donor syngeneic mouse
Receptor mouse
B
a
b
c

Figure 3

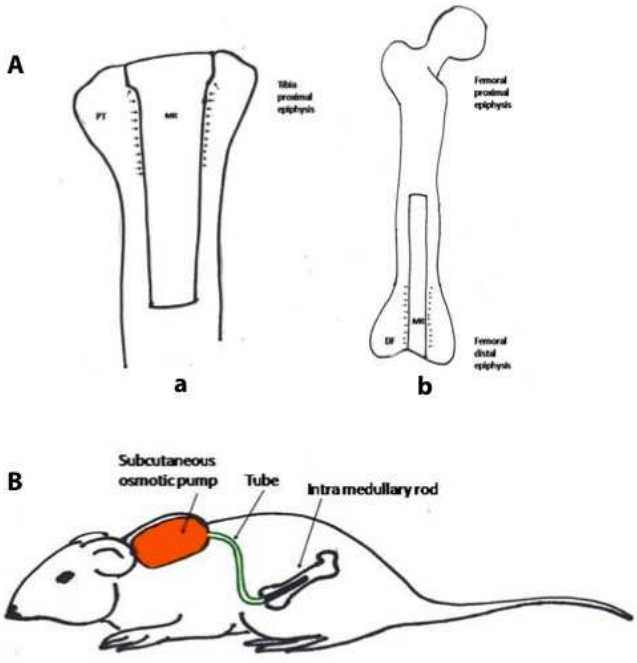
A
Tibia proximal epiphysis
Femoral proximal epiphysis
Femoral distal epiphysis
a
b
B
Subcutaneous osmotic pump
Tube
Intra medullary rod

Figure 4

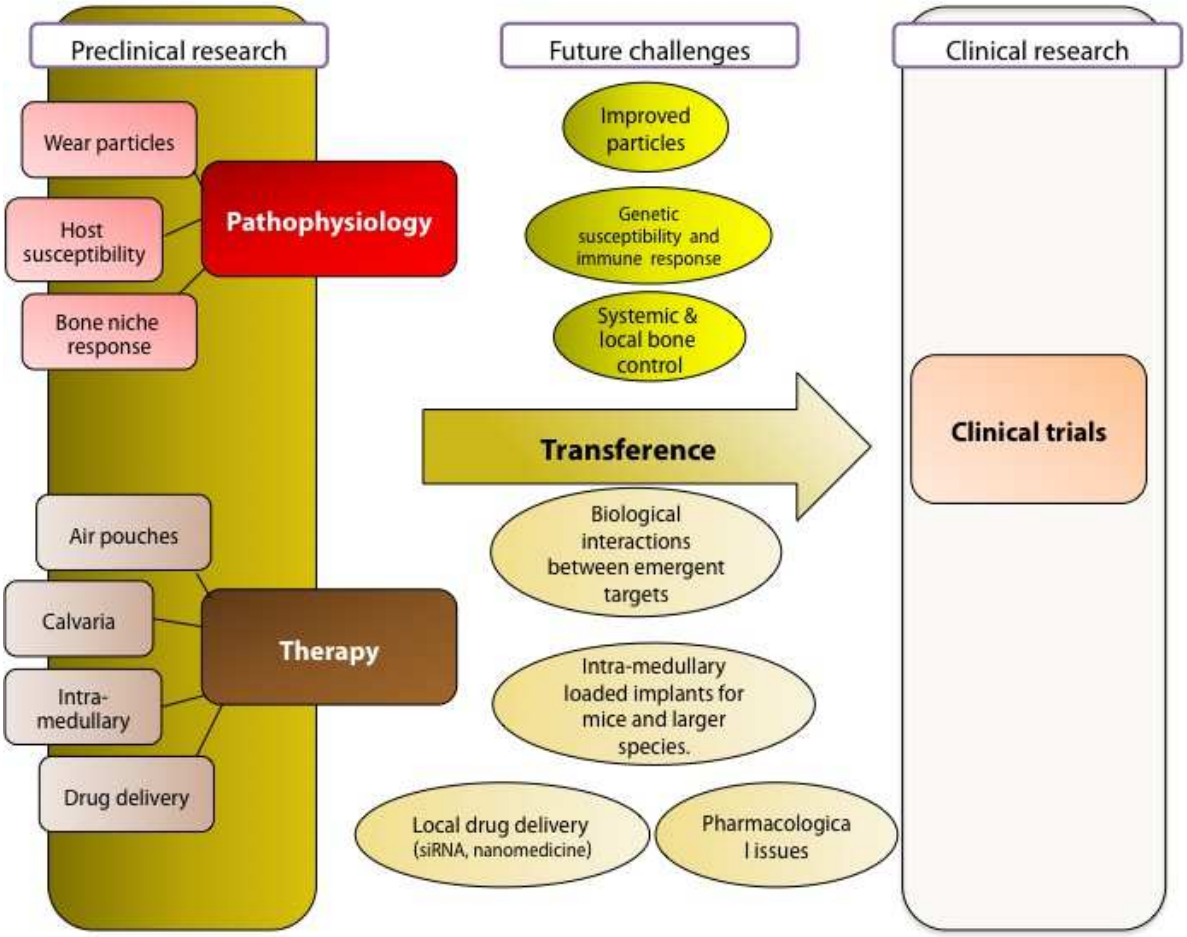
Preclinical research
Wear particles
Host susceptibility
Bone niche response
Pathophysiology
Air pouches
Calvaria
Intra-medullary
Drug delivery
Therapy
Future challenges
Improved particles
Genetic susceptibility and immune response
Systemic & local bone control
Transference
Biological interactions between emergent targets
Intra-medullary loaded implants for mice and larger species.
Local drug delivery (siRNA, nanomedicine)
Pharmacological issues
Clinical research
Clinical trials

Figure 5