Nano-carrier based drug delivery systems for sustained antimicrobial agent release from orthopaedic cementous material

by

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Abstract

Total joint replacement (TJR), such as hip and knee replacement, is a popular procedure worldwide. Prosthetic joint infections (PJI) after this procedure have been widely reported, where treatment of such infections is complex with high cost and prolonged hospital stay. In cemented arthroplasties, the use of antibiotic loaded bone cement (ALBC) is a standard practice for the prophylaxis and treatment of PJI. Recently, the development of bacterial resistance by pathogenic microorganisms against most commonly used antibiotics increased the interest in alternative approaches for antimicrobial delivery systems such as nanotechnology. This review summarises the efforts made to improve the antimicrobial properties of PMMA bone cements using nanotechnology based antibiotic and non-antibiotic delivery systems to overcome drawbacks of ALBC in the prophylaxis and treatment of PJIs after hip and knee replacement.

Keywords: PMMA, nanotechnology, bone cement, nanoparticles, antimicrobial, TJR.

Contents

1 Introduction

Total joint replacement (TJR), such as hip and knee replacements, are increasing worldwide because of growing aging population and risk factors such obesity. For example, more than 1 million hip and knee replacement are performed annually in the United States, while more than 160000 replacements were performed in the UK in 2014 only. Prosthetic joint infections (PJI) is a serious problem that is not only reduces success rate and need for revision surgery, but also leads to patient death. The treatment of such infections is complex with aggressive surgical intervention and long antimicrobial therapy, which places huge burden on health care systems worldwide.

The use of PMMA bone cement is considered the gold standard in hip and knee replacement, because of its mechanical performance and well-documented clinical history. PMMA bone cements major function is to fix the implant in adjacent bone, but also they are frequently used to release antibiotics for the prophylaxis and treatment of PJI. The use of antibiotics loaded PMMA bone cements is a standard practice in TJR with concomitant systemic antibiotics. Local release of antibiotics is preferred over systemic release, because of higher concentration of antibiotics are delivered locally avoiding side effects associated with systemic therapy.

Nowadays, antimicrobial resistance to many antibiotics decreased their efficacy in the treatment of infections, particularly PJI. This problem necessities the development of new antimicrobial agents to keep up with the emergence of bacterial strains resistant to currently used antibiotics. Nanotechnology have been applied successfully in the improving drug delivery in the treatment of many diseases such as cancer [1], inflammation [2], hypertension [3]. Therefore, nanotechnology can serve as an approach to solve the limitations of antimicrobial therapy and most importantly antimicrobial resistance by developing platforms for efficient drug delivery, and developing new antimicrobial nanomaterials which pathogens may not be able to develop resistance [4]. Nowadays, the development of antimicrobial resistance is much faster than the discovery of new antimicrobial agents, because of

occurrence of resistant bacterial strains and the long process for the approval of new drugs which increases the demand for long-term solution. This review introduces the use of nanotechnology in PMMA bone cements, especially in improving the antimicrobial properties and providing prophylaxis from PJI after hip and knee replacements.

2 Total joint replacement

The replacement of a dysfunctional joint with an orthopaedic implant is reserved as the last choice for the treatment of joint diseases. Arthritic and degenerative diseases is a leading cause of disability worldwide [5]. The most common form of arthritis is osteoarthritis which affects around 15% of the population [6]. In the United States (US), more than 26 million people are suffering from osteoarthritis [7], while that number reaches 8.5 million in the United Kingdom (UK) [8].

Total joint replacement (TJR) is the treatment of choice for patients with end-stage arthritis when less invasive therapies fail to alleviate the severe pain or dysfunction of the joint (Figure 1) [9]. This procedure showed noticeable progress in patients' quality of life [10,11]. According to the National Joint Registry [12], the predominant indication for TJR was osteoarthritis (more than 90% in hip and knee replacements) between the years 2003 and 2014. Whereas, a small percentage undertook TJR for other reasons, such as avascular necrosis, trauma infection and inflammatory arthritis.

The popularity of total hip and total knee replacements is increasing worldwide which places a huge burden on health care systems [13–15]. In the US, over 1 million hip and knee replacement are performed annually [16]. This number is expected to increase drastically in the next 20 years because of ageing as well as growing prevalence of risk factors such as obesity [17,18]. In the UK, the same trend is apparent; between the years 2003 and 2014 708,644 and 772,113 primary hip and knee replacements were performed, respectively. In 2014, 83,125 hip and 91,955 knee replacement were performed in the UK (Figure 1) [12].

2.1 Revision surgery

Despite the ability of TJR to improve the quality of life and retrieve mobility to many patients, the life expectancy for this procedure is around 10-15 years [19–21], hence there is a need for revision surgeries. Revision surgeries are the joint replacements performed after primary TJR because of implant failure. In the US, revision surgeries account for 18% of hip and 8% of knee total replacements performed each year [22]. Similarly, revisions in the UK are 11 % (8925) of hip and 6 % (5873) of knee primary procedures performed in 2014 [12]. The main reasons for revision are aseptic loosening, pain, and infection.

Compared to primary surgery, revision surgery is more complex and takes longer time to perform [23]. In addition, clinical and functional outcomes are poorer such as pain, joint stiffness and stability, muscle impairment and atrophy, with lower patient satisfaction and quality of life after surgery, because of complexity and nature of revision surgery [24]. Thus, revision is accompanied by higher complication rates, longer patient hospital stay and the use of a more expensive implants [25,26]. Accordingly, revision surgeries are associated with higher costs when compared to primary replacements, as well as relatively shorter survival [27]. For example, the cost of primary knee replacement is around $15000, while the cost of revision surgery is higher and can reach $ 24000 [26]. In the UK, health care costs for revision surgery were estimated to be 80 million in the year 2010 [28].

Infection after joint replacements is a severe problem that not only decreases the success rates of surgery, but also can be life threatening to patients. Despite antibiotic prophylaxis and operation under laminar flow, infection rates in the first two years of primary replacement are 1% in knee replacements, 2% in hip replacements and can reach 9% in other types of TJRs. Also, infection rates are significantly higher after revision surgeries (up to 40%) [29,30]. These percentages translate into large numbers when we look at the total numbers of TJRs done annually. For example, 2,400 revision procedures were performed in the UK in 2014 due to infection [12] and 22,000 revisions of infected knee and hip replacements were done in the US in 2009 [31]. Prosthetic infections extend hospitalization time, readmissions

and length of antimicrobial treatment, hence increasing the economic burden on health care systems; the cost of treatment for an incident of prosthetic infection can reach $50000 which is more than 3 times the cost of primary surgery and 2 times the cost of revision surgery [32].

2.2 Cemented joint replacements

Nowadays, there are two main types for TJRs, namely, cemented and cementless joint replacements (Figure 2). In cemented TJRs, bone cement is widely used for fixation of prosthesis. Poly(methyl methacrylate) (PMMA) based bone cement is the gold standard material used in such procedures. This type of TJR involves complete removal of the impaired joint, after that a cavity is made inside the bone. The surgeon fills the cavity with PMMA bone cement. Then, the metallic implant is placed and positioned in the cavity while the cement sets. Cementless TJRs follow the same procedure except that the implant is inserted in direct contact with bone without using a cement [33].

Bone cements are routinely used in TJRs to fasten the orthopaedic implant in place; transfer mechanical stresses and loads between the stiff metallic implant and bone tissue; and, commonly, to provide prophylaxis from post-surgical prosthetic infections by releasing one or more antibiotic such gentamicin or tobramycin (Figure 3) [34]. In addition, advantages for using bone cement include that the bone cavity does not have to be perfect match with the implant and the use of bone cement reduces the need for blood transfusions, because of reduced blood loss and the cement tamponade effect [35,36]. Furthermore, the most important reason for using PMMA bone cement in TJR is the outstanding long term survivorship (98% at ten years and 91% at 20 years) [37,38]. However, there are always concerns about cemented replacements because of their degradation products and debris, as well as deterioration of bone cement interface and third body wear [39]. These concerns led researchers to seek new alternatives for fixation, i.e. cementless fixation.

Cementless replacements depend on biological fixation or osteo-integration of the implant to the bone; advocates of this type of fixation believe that bone ingrowth through the micropores of the metallic implant can achieve more durable fixation with bone (Figure 2b).

The claimed advantages of cementless fixation are: shorter operation time, ease of revision, and improved longevity for active younger patients [40]. However, cementless fixation has inconsistent long term results and is not regularly used in most centres, because it is accompanied with a high rate of revision [12,41,42].

2.2.1 Total knee replacement

The use of cemented implant is the 'gold standard' in total knee replacement (TKR) in the last 3 decades and has high success rates of more than 95% at 15 years with long term durability [37]. Many articles reported outstanding long term results for cemented TKR. Crowder *et al.* (2005) analysed 32 patients with cemented implants, he reported survivorship rate of 100% in 15 years and 93.7% in 20 years after TKR procedure. Gill *et al.* (1997) [44] reported 96.5 % survivorship 18 years after the procedure in patients 55 years old or younger. Ritter *et al.* (2007) [45] also reported 97.6% success rate in the same age group when followed for 9.1 years. Vessely *et al.* (2006) [46] looked at 244 patients with cemented TKR, survivorship was 95.7% in 15 years after the procedure. Another study, including 265 patients with posterior stabilized prosthesis, had 94.1% success rate over 16 years [47].

Many authors have directly compared cemented fixation with cementless fixation in TKR [48,49]. Rand *et al.* (2003) [50] carried out a survivorship analysis for 11606 patients at 10 years. The success rate was 92% in patients with cemented prostheses, whereas only 61% success rate reported in patients without cement ($P<0.0001$). Barrack *et al.* (2004) [48] compared 82 cementless mobile bearing knees with 73 cemented knees, 8% of cementless knees were revised, while no revision found in cemented knees. Rorabeck (1999) [12] looked at 484 patients of hybrid and cemented knee fixation, reporting 9.6% revision rate in hybrid group (uncemented femur and cemented tibia), compared to 1.6% in the cemented group after 3 years [49]. Figure 4 shows the number of TKR in UK between the years 2003-2014.

2.2.2 Total hip replacement

Clinical studies performed on total hip replacement (THR) with cemented implants have convincing long term results. Berry *et al.* (2002) [51] reported a survivorship rate of 80% in 25 years after the procedure in 1689 patients with cemented implant. Another study about cemented implant including 226 patients reported similar survival rate of 81% in 25 years [52]. However, a tendency towards cementless hip replacement has been seen in recent days, because of the significant improvement in survival rate for cementless stems. In patients using cementless BiCONTACT stem, the survival rate is 94.4% in 15 years [53]. Emerson *et al.* (2002) [54] looked at 181 patients with cemented and cementless hip implant. The survivorship was 84 % in cemented group, while it was 100% in cementless group. Cementless implants are specifically selected for young active patients who have greater physical loads with greater failure rates secondary to loosening, whereas cemented implants are used for older patients with poor bone quality [55,56]. Figure 5 shows the number of THR in the UK between the years 2003-2014 [12].

2.3 PMMA bone cements

Poly (methyl methacrylate) polymer (PMMA) is a polymer based on methyl methacrylate (MMA) monomer units. PMMA cement is prepared by mixing two constituents together: PMMA polymer powder and liquid MMA monomer (Figure 6). After mixing the two components, the hardened bone cement is formed by an exothermic free radical polymerization reaction, as the liquid monomer polymerizes around the pre-polymerized powder producing heat [57]. The heat of the setting reaction can reach (66-82.5 ^{o}C). The setting time for the cement is relatively short (less than 15 min) and the cement must be inserted into the bone before cement hardening, otherwise the procedure cannot be completed [58]. Premature polymerization of the liquid component may happen because of exposure to heat and light. Therefore, Hydroquinone is added as a stabilizer to prevent polymerization before mixing of the cement constituents. Benzoyl peroxide is added to the powder to initiate the free radical polymerization reaction, while N, N-Dimethyl para-toluidine

(DMPT) is added as an accelerator to facilitate the polymerization reaction between the polymer and monomer at room temperature (Chaudhry & Dunlop, 2012). Barium sulphate ($BaSO_4$) or zirconium dioxide (ZrO_2) are added as radiopaque agent to allow X-ray imaging because PMMA is not radiopaque [59].

At present, many commercial bone cements are marketed by different manufacturers (Table 1). The main differences between different formulations are the molecular weight of PMMA, the ratio between homopoylmer and copolymer, the ratio between powder and liquid, the radiopacifier and other additives such as antibiotics. Different copolymers of different acrylic monomers are added to modify the mechanical properties of the cement, such as MMA, styrene and Ethyl methacrylate [60]. Table 1 shows the composition of some commercially available PMMA bone cements.

The main drawback of PMMA bone cement is the absence of bone bonding ability, i.e. bioactivity. This can lead to the formation of fibrous tissue around the implant and a space for the wear particles to accumulate [61]. As a result, bone resorption around the implant causes loosening and failure of the implant after a long period of time, which is the most commonly reported reason for revision in cemented replacements [12]. Despite the extensive research done on developing alternatives for PMMA in THR and TKR, PMMA stays to be the biomaterial of choice in TJRs since the 1960s, because of its acceptable long term survivorship and long-established clinical history as well as excellent mechanical properties [57]. Extensive research has been directed on developing new bioactive bone cements that integrate with bone, and improving the biocompatibility as well as mechanical properties of PMMA bone cements [62,63].

One of the examples on bone cements with bioactive properties is calcium phosphate bone cement (CPC), which has been studied since 1980s [64]. Their poor mechanical properties such as strength, toughness and brittleness limited their application to low load-bearing arthroplasties e.g. craniofacial and maxillofacial surgeries (Table 2). Despite CPCs bioactive properties, their inferior mechanical properties are not sufficient to replace the use of PMMA

in high load-bearing arthroplasties such as knee and hip replacements [65,66]. The mechanism for setting reaction involves a dissolution-precipitation process that occurs at body temperature, without causing tissue necrosis in the surrounding tissue unlike the exothermic setting reaction for PMMA [67]. Despite the presence of many CPC formulations, the final product only could be either brushite or hydroxyapatite. Brushite is a metastable form that may transform into hydroxyapatite at pH>4 in vivo [68]. CPCs are microporous in nature which helps in the penetration of biological fluids, hence they are resorbable and can be replaced by bone [69]. In addition, the micropores enhance the ability of CPCs to load drugs which is an appealing option for any type of biomaterial [70,71].

Apatite/wollastonite is another bioactive bone cement that has been researched for use in knee and hip replacements. Apatite/wollastonite glass bioactive ceramics have currently many medical applications and used as bone filler or bulk material [72]. Also, they have higher mechanical properties than other bioactive ceramics and cortical bone (Table 2). However, they cannot be used in high load arthroplasties such as hip and knee replacements, because their fracture toughness is lesser and elastic modulus is greater than those of cortical bone [73].

Dental cements have been also researched for orthopaedic application such as glass polyalkenoate and Bioglass [74,75]. Glass polyalkenoate is a dental cement with good mechanical properties (Table 2), but the release of aluminium from the glass phase causes defective bone mineralization and limits their use in the orthopaedic field [76]. In order to avoid this problem aluminium was replaced with Zn, as it has a positive effect on osteoblast proliferation and increases bone mass. However, Zn based glass polyalkenoate has substantially inferior mechanical and setting properties compared with aluminium containing counterparts [75]. Moreover, resin modified glass polyalkenoate, another biomaterial, was developed to improve the poor mechanical properties of conventional glass polyalkenoate. Although it has good mechanical properties, they suffer from volumetric shrinkage after curing which causes mechanical failure at the implant interface [77,78].

None of the previously mentioned bioactive cements have the required mechanical properties to be used in high load bearing arthroplasties. Despite the lack of bone-bonding properties of PMMA, it is still the only biomaterial to be used in cemented hip and knee arthroplasties. Therefore, PMMA fails to achieve a long-lasting replacement making aseptic loosening the most common cause for revision. Newly developed bone cement should have both bone-bonding properties (bioactivity), as well as mechanical properties that match those of bone and optimally have antimicrobial properties [79].

2.4 Prosthetic infections

The success of TJRs in relieving pain and improving the quality of life for patients is increasingly growing. Infection is considered the most serious problem after joint prosthesis implantation, which decreases success rate of the surgery and can be life threatening to patients in some cases [29]. Prosthetic infections are difficult to diagnose and occur at variable times after the primary surgery. Management of prosthetic infections is complex and needs multiple procedures and prolonged antimicrobial therapy with poor functional outcome [30]. This places considerable burden on medical resources and health care expenditure, because of the high cost of prosthetic joint infection incidence treatment that can reach up to $50000 [32]. Efforts have been made to reduce the risk of prosthetic infections such as the use of perioperative antimicrobial prophylaxis and surgical laminar airflow environment, however the incidence of prosthetic infection is still high and can reach up to 2% in total hip and knee replacement, and even higher after revision surgeries (up to 40%) [29].

Prosthetic infections have 3 classifications based on the onset of infection, namely, (i) early, (ii) delayed, (iii) late infections. For early infections, the signs and symptoms of infections appear in the first 3 months after surgery, and the infection are usually because of bacterial contamination during or after surgery caused by highly virulent microorganisms. Early infections account for up to 45% of prosthetic infections. In delayed infections, the first signs and symptoms appear after 3 months to 2 years after surgery. The causes of delayed infections are low virulent microorganisms inoculated during surgery. In late infections, the

onset starts after 2 years from surgery, and caused by seeding via the blood from an infection in other body parts such as skin, respiratory or urinary tract infections [80–82].

Biofilm formation is the typical mode of growth for bacteria involved in prosthetic infections, which adds to the difficulty and length of treatment. The microorganisms in biofilms form ordered and complex clusters enclosed by a hydrophilic polymeric matrix [30,80]. Biofilms shelter microorganisms form antibiotics and host immune defence, as well as increase bacterial resistance and reduce susceptibility to antibiotics by 500-5000 times compared to planktonic, free floating bacteria [29]. In addition, the implant acts as a binding site for bacterial accumulation into biofilms and decreases the minimum dose of bacteria needed to cause infection [30].

The most commonly encountered bacteria in prosthetic joint infections are coagulase-negative staphylococci (30-43%) and *Staphylococcus aureus* (12-23%), followed by streptococci (9-10%), Gram-negative bacilli (3-6%), enterococci (3-7%), and anaerobes (2-4%). Polymicrobial infections, which usually occur postoperatively, are seen in (10-12%) and they are difficult to treat.[80,83].

2.4.1 Treatment

The treatment of prosthetic infection aims to relieve patients from pain, restore joint mobility and eradicate infections. Treatment of such infections is typically challenging and complex with combined aggressive surgical interventions and antimicrobial therapy, which make it hard to achieve all of the 3 aims together. Management of prosthetic infections should be customised for each patient and usually includes one of 3 main types of surgical interventions [29,84]. First, prosthetic retention with debridement of all infected tissue and irrigation, which is a choice for early postoperative or late haematogenous infections with retention of the prosthesis and long term antibiotic treatment [85,86]. Second, prosthetic exchange, the most frequently used, by one stage or two stage revision. In one stage revision, the removal of all foreign material debridement and reimplantation of a new prosthesis are done in the same procedure [87]. While in two stages revision, the removal of

foreign material and debridement are done, and the reimplantation of a new prosthesis is delayed for a variable period of time (typically after > 6 weeks) [88]. Third, salvage procedure including resection arthroplasty, arthrodesis and amputation which are the last choice when infection management is not achievable by the previously mentioned interventions [89].

Two stage revision has become the standard procedure in the treatment of deep tissue prosthetic infections [90]. The two-stage approach gives sufficient time for debridement and removal of the infected tissues, the determination of the infecting microorganism and its sensitivity to antibiotics, modifying the antimicrobial therapy before reimplantation. However, extended hospitalization increases the surgery costs, while delayed mobilization and risk of other surgery is cautiously considered, particularly in elderly people [91].

In two stage surgery, the use of antibiotic-impregnated spacers is considered the gold standard for the eradication of infection and avoiding limb shortening [92,93]. Spacers are bone cement pieces that is placed in the joint place to prevent muscle contractions and preserve their length. The use of a temporary spacer in two-stage surgery in knee replacement gives the patient the ability to move, also provides good alignment of the knee between the two stages [94,95]. Success rates with the use of antibiotic impregnated PMMA interim spacer/prosthesis are reported to be higher than 90% [96]. The advantage offered by such spacer is delivering high level of antibiotic locally, while maintaining joint mobility [97]. Table 3 shows common antibiotic combinations used for the impregnation of PMMA bone cement spacers.

2.4.2 Prophylaxis

Antibiotic loaded bone cements (ALBC)s are routinely used in hip and knee TJRs not only in the treatment of prosthetic infections, but also to prevent infections after cemented replacements, and their use become a well-established practise along with peri-operative systemic antibiotics [98,99]. More than 90% of surgeons use ALBC in primary TKR in the UK [100], Sweden [101], and Norway [99]. The use of ALBC in knee replacements reduces the percentage of prosthetic infection compared to bone cements lacking antibiotics [102,103]. Similarly, the use of ALBC in hip replacements improves survivorship by reducing the risk of prosthetic infections after primary replacements [104,105]. A meta-analysis evaluating the efficacy of ALBC in hip replacements reported that the use of ALBC reduces prosthetic infections after primary hip replacements from 2.3% to 1.2%, and 40% after revision [98].

Local antibiotic release from the bone cement gives higher concentration in the joint compared with systemic antibiotics, which are hindered by limited blood circulation at the site of implantation [106,107]. Moreover, local delivery of antibiotics avoids the adverse effects of high antibiotic levels in the blood, such as nephrotoxicity and ototoxicity [108]. Hence, ALBC provide an alternative strategy for the prosthetic infection prophylaxis.

The antibiotic loaded acrylic bone cements available commercially can be either a premixed powder, where the antibiotic is blended with the cement powder by the manufacturer, or an off-label formulation. In off-label formulations the antibiotic powder is provided separately to be mixed with the cement by the surgeon during surgery [109,110]. Low concentrations of the antibiotic (0.5-1.0 g per 40 of powder) are used for primary arthroplasty prophylaxis and second stage of a two-stage revision arthroplasty, while high concentrations (2.0-4.0 g per 40 g powder) are used for the treatment of existing active infection [111].

The choice of antibiotic for incorporation in the bone cement depends on several factors. Desirable antibiotic characteristics include availability in powder form, wide antibacterial spectrum, thermal stability to withstand the high exothermic temperature of the setting reaction, elution from the bone cement for a prolonged period, low allergic effects and most

importantly low influence on the mechanical properties of the bone cement [112]. Among the antibiotics used, which usually meet these criteria, are aminoglycoside (gentamicin and tobramycin) [113] and glycopeptides (vancomycin) [114]. The combination of antibiotics from more than one group gives a wide antimicrobial spectrum [115]. Table 4 shows some of the commercially available bone cement brands and the incorporated antibiotics.

2.5 Limitations for antibiotic loaded bone cements

2.5.1 Antibiotic elution properties from bone cement

The elution kinetics of antibiotics from PMMA bone cements are highly variable and depend on many factors. Different brands of bone cements come with different compositions, viscosities and porosities [116,117]. Hence this leads to differences in their ability to release antibiotics. Porosity is introduced into the cement by the formation of air bubbles during the exothermic setting reaction and depends on the viscosity and manipulation technique [118]. Porosity increases antibiotic elution from bone cement but at the same time has a negative impact on it's mechanical properties [119]. Among other factors affecting elution kinetics is a type of antibiotic used or antibiotic combinations [115,120].

The ideal ALBC should sustain the release of antibiotic at high concentrations for a long time to prevent early onset infections and avoid the development of resistant bacterial strains [112]. However, the antibiotic release from ALBC, in reality, is characterised by initial uncontrolled burst release for the first few hours after surgery. Subsequently, the antibiotic release drops rapidly below inhibitory levels within few days, and does not provide long term sustained delivery of antibiotics [121–124]. Moreover, more than 90% of the loaded antibiotic may still be entrapped within the hydrophobic PMMA matrix [125,126]. The initial burst release occurs when the ALBC is exposed to fluid surrounding the joint and governs mainly by a surface phenomenon because of the presence of antibiotic agglomerates on the

surface of bone cement, while the sustained release over the next few days is a bulk phenomenon and more affected by the porosity of cement [127].

2.5.2 Development of antimicrobial resistance

Antibiotic burst release from the bone cement is followed by slow release of antibiotic at low concentrations below the minimum inhibitory concentration needed to kill bacteria [122,124]. This slow release increases the chances for selecting resistant microbial strains which raises concerns about future effectiveness of antibiotics used in ALBCs [128,129]. The bacterial strains selected at low antibiotic concentrations are generally highly resistant [130]. Some experimental studies show the capacity of pathogens to grow on the surface of ABLC and the ability to form biofilms [30,127]. Anguita-Alonso *et al.* (2005) investigated the susceptibility of Staphylococci taken from patients with prosthetic infection against gentamicin and tobramycin (aminoglycoside antibiotics) [131]. 41% and 66% of bacteria were resistant to gentamicin and tobramycin respectively. Corona *et al.* (2014) compared antibiotic susceptibility between patients having infection for the first time and patients with previous use of ALBC and found a significantly higher resistance, indicating the risk of selecting aminoglycosides resistant strains after using ALBC [132].

2.5.3 Antibiotics effect on the mechanical properties of bone cement

Addition of antibiotics to bone cements has a negative impact on their mechanical properties. Small quantities of antibiotics (< 1g per 40 g of bone cement) slightly decrease compressive and bending strength of bone cement but stays in the acceptable range stated by the standard ISO 5833:2002, while high antibiotic quantities cause a significant decrease in the mechanical properties [110,133]. The acceptable ranges for the mechanical properties of a set bone cement are > 70 MPa compressive strength, > 1800 MPa bending modulus and >50 MPa bending strength [134]. High dose ALBCs (>2g per 40g cement) are only used temporarily in spacers for the treatment of prosthetic infection in two stage surgery, because their poor mechanical properties, while low dose ALBCs (< 2g per 40g cement) are used for prophylaxis where mechanical properties are important for implant fixation [121,135].

Persson *et al.* (2006) reported a detrimental decrease in the bending (-22%) and fatigue strength (-15%) of bone cement when vancomycin was added at 2.5% w/w [136]. He *et al.* observed that the use of gentamicin at concentrations below 3% had no significant effect on the compressive and elastic modulus of bone cement; however, higher concentrations caused significant decrease in these two parameters [137].

3 Novel bone cement formulations

3.1 Role of nanotechnology

Currently used antibiotics have many limitations including microbial resistance, narrow therapeutic index, cytotoxicity and side effects linked to non-selectivity in their mode of action and poor release profiles from carrier systems. Nanotechnology, which refers to the production and application of materials in the size range (1-100nm), has been used in the treatment of many diseases such as cancer [1], inflammation [2], hypertension [3]. The success of nanotechnology in improving drug release in the treatment of many diseases makes it an appealing approach for application in antimicrobial therapy. Nowadays, the development of antimicrobial resistance is rapidly increasing compared to the discovery of new antimicrobial agents. Therefore, the development of nanotechnology drug delivery systems or new antimicrobial nanomaterials can be used to overcome the problems of inefficient delivery of antimicrobial agents and resistance to currently used antimicrobials [4].

Novel nanotechnology drug delivery systems offer many advantages to overcome the current challenges with antimicrobial therapy. Nanoparticles have unique physicochemical properties such as large ratio of surface area to mass, small size, and ease of structural or functional modification. The antibiotics can be loaded into nanoparticles by physical encapsulation, adsorption or chemical conjugation where the drug release profiles can be significantly altered compared to free drug counterpart, enhancing poor delivery of drugs and sustaining release [138]. In addition, specific microbial resistance mechanisms to antibiotics can be overcome using nano-systems, which act on multiple biological pathways present in

most types of bacteria [139]. Moreover, nano-carriers can be used for the delivery of multiple antibiotics to provide synergistic effect against resistant strains [140]. Nanoparticles labelled antibiotics increase binding to bacteria and the concentration at the site of infection. These improvements can be attributed to the enhanced solubility of drugs and controlled release profiles. Also, nano-systems decreases side effects by enhancing cellular internalization and uniform distribution in the target tissue, and improving the pharmacokinetic profiles and patient compliance to antibiotics [141]. Compared to antibiotic synthesis, the preparation of nanoparticles is cost-effective giving stable formulations for long term storage. Although, antibiotics can be degraded easily at harsh conditions, nanoparticles can withstand harsh conditions such as high temperature and sterilization [4].

3.2 Nanotechnology based antibiotic based antimicrobial bone cements

Nanotechnology based antibiotic delivery systems is a becoming a new approach for solving the limitation of antimicrobial therapy. Nanoparticles can be used to improve the release kinetics of antibiotics by enhancing delivery and providing controlled release. These improvements are attributed to large area to mass ration and small size, and different ways available for modification and for antibiotic loading [138]. Many nanotechnology-based antibiotic carriers have been researched to improve the antibiotic release profile from PMMA bone cement including liposomes [142], mesoporous silica [143], carbon nano-tubes, hydroxyapatite nano-rods and clay nanotubes [144].

Although liposomes have miscibility problems in non-aqueous environment because of their hydrophilic surface, they were used to improve gentamicin distribution within PMMA bone cement. Liposomes have been largely used as drug carrier in aqueous suspensions, and have miscibility problems when mixed with PMMA leading to phase separation [145]. Ayre *et al.* (2015) [142] solved the problem of phase separation using Pluronics on the surface of liposomes (Figure 7).

Pluronics are surfactants made of interconnected chains of polyethylene oxide (PEO) and poly propylene oxide (PPO) subunits. It is hypothesized that the hydrophilic PEO will attach to the hydrophilic surface of liposomes, while the PPO will attach to the hydrophobic matrix of PMMA. Liposomes were suspended to the liquid MMA part of before mixing with PMMA powder. Moreover, pelleted liposomes of 100 nm size were prepared by extrusion and ultra-centrifuged with 3 different Pluronic surfactants (L31, L43, and L61). Gentamicin release from liposomal bone cement was sustained for 30 days with 22% of the loaded antibiotic released compared to 9% from commercial formulation. Gentamicin release was characterized by bust release in the first 72 hrs for commercial bone cement, while liposomal cement showed nearly linear release profile. Despite the slight reduction in compressive strength, the liposomal formulation enhanced the toughness, bending strength and Vickers hardness of cement when compared to Palacos R+G. The addition of liposomes improved the dispersion of gentamicin in bone cement and improved the mechanical properties as well.

In another work, Shen *et al.* (2016) [143] mesoporous silica nanoparticles (MSN) were used to improve the release kinetics of gentamicin from PMMA bone cement. The presence of 10% MSN enhanced the release for more than 60% of loaded gentamicin over 80 days. Furthermore, the concentration of MSN was found to be crucial to build a nano network to facilitate the diffusion of gentamicin molecules as supported by images (Figure 8). Hence, MSN concentration below 6 % could not improve gentamicin release. The compressive strengths of MSN functionalized bone cements is nearly the same as the commercial bone cement. However, the bending modulus is reduced by 10%. Moreover, the 10% MSN bone cement was cytocompatible with 3T3 mouse fibroblasts, showing 96% cell viability in 3T3 mouse.

Carbon nanotubes (CNT) were also tested for enhancing gentamicin release from PMMA bone cement. Although 5% (CNT) loaded bone cement lead to 75% release of gentamicin for 60 days, the compressive strength is reduced by 90% compared with the commercial

bone cement. Furthermore, CNT showed high toxicity to 3T3 mouse fibroblasts with 85% cell viability. Cytotoxicity of CNT is among the most concerns for its application in biological systems and it has also attracted more attention in recent investigation [147]. In the same work, hydroxyapatite nano-rods (HAP) were loaded with gentamicin by wet impregnation and loaded into PMMA bone cement at 32% concentration. At this concentration, 75% gentamicin was released over 60 days. Despite low cytotoxicity of HAP, as it is one of major compositions of bone structure, the compressive strength is decreased by 50% compared to the commercial bone cement.

In another study, clay nanotubes Halloysite is used to improve gentamicin release from PMMA bone cement [144]. Halloysite is a naturally occurring nanotube with a length of 500–1000 nm, diameter of 50 nm, and lumen of 15 nm. Therefore, it is highly biocompatible as confirmed by blue cell essays on HeLa and MCF-7 cell lines [148]. PMMA bone cement was loaded with 5-8% Halloysite and with 10-15% gentamicin. The release profile was characterised by burst in the first few days. After that, gentamicin release slowly continued for 250 hours. Furthermore, the addition of 5-7% Halloysite nanotubes improves the tensile strength and adhesive properties, except for flexural strength which is slightly reduced with higher concentration such as is 5% which gives both higher tensile strength and good flexural properties. Table 5 summarizes the mechanical properties of previously mentioned nanocomposites and Table 6 is a list of different nanotechnology based antibiotic loaded PMMA bone cements.

3.3 Non-antibiotic based antimicrobial bone cements

Quaternary ammonium compounds attracted research because of their antimicrobial properties and stable structure [149]. Chitosan quaternary ammonium nanoparticles impregnated bone cement showed antimicrobial activity against viable bacterial at a concentration of 15% w/w [150]. In another study, hydroxypropyl trimethyl ammonium chloride chitosan loaded (HACC) bone cement inhibited biofilms caused by methicillin-resistant Staphylococcus strains showing *in vitro* release for 120 hours [151], with enhanced

physical and osteogenic properties [152]. HACC-loaded bone cement was further evaluated in *vivo* for the treatment of Methicillin-resistant *Staphylococcus epidermidis* infection of the tibial metaphysis in a rabbit model, and exhibited effectiveness in the inhibition of bone infections [153]. One quaternary ammonium dendrimer of tripropylene glycol diacrylate (TPGDA) was mixed with bone cement at a concentration of 10%. At this concentration, TPGDA modified bone cement showed antimicrobial activity for 30 days. In addition, the dendrimer bone cement composite was potent to kill 108 CFU/mL of bacteria on regular intervals of 5 days for a month. However, the addition of dendrimer resulted in a reduction of compressive strength (>15%) compared to the original sample. Furthermore, the MTT assay for the dendrimer modified bone cement showed 12.5% reduction in the viable cells compared to the control, and cytotoxicity needs to be further determined [149]. Table 7 summarizes some examples of antimicrobial bone cements with potential application in total joint arthroplasties.

Quaternary ammonium chitosan derivative nanoparticles (QCS) achieved a 103-fold reduction in the number of viable bacterial cells upon contact with the surface when added at concentration of 15% to bone cement. Chitosan in the form of nanoparticles is better in preserving the mechanical properties of the bone cement compared to powdered chitosan, i.e. Young modulus and bending modulus is >90% of the original bone cement values. When the CS (powder not NP) loading was decreased to 15%, the Young's modulus and bending modulus are about 90% of the corresponding properties of the original bone cement. This can be explained by the homogenous distribution of nanoparticles inside the bone cement matrix, which minimizes the macroscopic cracks in cement mantle. QCS nanoparticles showed higher antimicrobial activity compared to chitosan nanoparticles at the same concentration, where the viable cell number declined by about three orders and two orders of magnitude, respectively. However, The MTT assay showed that there is no significant difference in cytotoxicity between the CS NP, QCS NP and the non-toxic control [150].

Silver nanoparticles have many applications in medical field as safe and effective antimicrobial agents, such as bandages, catheters and surgical scrubs. However, systemic administration of silver nanoparticles can cause various health problems when it reaches toxic levels in different body organs [154]. Consequently, local delivery of silver may decrease the adverse effects of high silver levels in the blood. Oei *et al.* (2012) [155] investigated the antimicrobial properties of a PMMA bone cement impregnated with silver nanoparticles. Despite in *vitro* release of silver ions for 28 days and broad spectrum antimicrobial activity, the mechanical properties of bone cement was negatively affected at the concentration used (1% w/w) and showed lower bending modulus. Silver nanoparticles prepared with different capping agents were studied for bone cement impregnation. Prokopovich *et al.* (2015) [156], reported a broad spectrum antimicrobial activity of silver nanoparticles capped with oleic acid at low concentrations of 0.05 w/w %, without affecting the mechanical properties and cytotoxicity of the bone cement. Similar preferable antimicrobial and mechanical properties were identified when silver nanoparticles capped with tiopronin were impregnated in PMMA bone cement at a concentration of 0.1 w/w % [157].

In another study, Perni *et al.* (2015) [158] developed a propyl paraben nanoparticle loaded bone cement at a concentration of 7% w/w. Nanoparticles at this concentration exhibited wide spectrum antimicrobial killing with no detrimental effect on mechanical properties and cytocompatibility.

4 Drug delivery systems and nano-formulations for potential use in bone cements

Some approaches to prolong drug release have been conducted but efficacy has not been tested in bone cement yet (Table 8). One of the novel approaches for enhancing the delivery of aminoglycoside antibiotics is Layer by Layer assembly (LbL). LbL has numerous applications in drug delivery [159]. This coating technique is a versatile method and involves

the deposition of alternative oppositely charged polyelectrolytes on different substrates, allowing control of the thickness and composition of coating at nanoscale level in a reproducible manner [160,161]. Tamanna *et al.* (2015) [162] managed to control the release of gentamicin from gentamicin loaded mesoporous silica nanoparticles coated using LbL technique. The coating polyelectrolytes were polystyrene sulfonate (PSS) and poly (allylamine hydrochloride) (PAH). The coated layer controlled drug release for 10 days with no burst release compared to the same gentamicin loaded nanoparticles without coating. In another work, Lichter & Rubner (2016) [163] developed an antimicrobial LbL assembly without the addition of biocidal species, by optimizing the conditions during and after layers deposition in order to expose the cationic charges needed for antimicrobial activity. Multilayers of PAH, PSS and poly(acrylic acid) (PAA) were constructed at high pH and subsequently immersed in low pH solutions, which showed antimicrobial activity against *S. epidermidis* and *E. coli*. In a similar work, Kovačević *et. al*. (2016) [164] studied the changes in surface material properties by using LbL coating, and their effect on the bacterial adhesion of *P. aeruginosa*. Multilayers of PAH and PSS were built on the silica surface, where the polyelectrolyte multilayers terminating with negatively charged polyelectrolyte showed less bacterial adhesion on the surface.

Mu *et al.* (2016) [165] evaluated the antimicrobial properties of phosphatidylcholine-decorated Au nanoparticles loaded with gentamicin (size 180 nm), which showed broad spectrum activity and inhibition of biofilm formation. The presence of phosphatidylcholine on the surface facilitated the electrostatic binding of gentamicin. The nanoparticles were more efficient in the inhibition of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilm, when compared to gentamicin or phosphatidylcholine Au nanoparticles without gentamicin. Gentamicin release continued for 7 days in buffer media pH 7.4, and the loading efficiency was 38µg/ml (gentamicin/Au). Cytocompatibility studies were done using RAW 264.7 cells and the nanoparticles were nontoxic and can be engulfed by macrophages.

Fan *et al.* (2016) [166] loaded chlorhexidine on Ca-silicate mesoporous nanoparticles (size 78.6 nm) using mixing-coupling technique. The nanoparticles were able to release chlorhexidine as well as Ca^{2+} and $silicate^{2-}$ ions for up to 9 days in simulated body fluids. They showed antimicrobial activity against *Enterococcus faecalis* which is commonly reported to be involved in root canal infection. The nanoparticles did not show any negative effect on cell proliferation and showed in *vivo* mineralization effect, which give them the potential to be used in intra-canal defects or bone infections.

Poly (lactide-co-glycide) (PLGA) is hydrophobic biodegradable and biocompatible polymer that is approved for clinical use. Abdelgahany *et al.* (2012) [167] prepared gentamicin PLGA nanoparticles through emulsion evaporation method, using two approaches: water/oil/water and solid/oil/water. The size for the nanoparticles were 251 nm and 359 nm, respectively, with loading efficiency reached up to 22.4 µg/ml. Gentamicin release from the nanoparticles continued for up to 16 days at pH 7.4. In addition, the nanoparticles showed antimicrobial activity against *P. aeruginosa* planktonic bacteria and biofilms, as well as in *vivo* infected mice model.

Kurtjak *et al.* (2016) [168] loaded gallium nanoparticles (size 22nm) into hydroxyapatite nano-rods bioactive composite through ultrasonic emulsification. The gallium nanocomposite showed better antimicrobial properties against *Pseudomonas aeruginosa*, when compared to silver nanocomposite, as illustrated by microdilution assay and MIC determination. Also, gallium nanoparticles had lower toxicity for human lung fibroblast and mouse fibroblasts.

5 Conclusion

The currently used ALBCs have many limitations in terms of antimicrobial performance and elution of antibiotics from PMMA matrix. In addition, there is a need for the development of new antimicrobial agents and antibiotic delivery systems to overcome the emergence of resistant bacterial strains encountered in PJIs. The use of nanotechnology in antimicrobial

treatment is attracting more attention in literature. As a result, its application in ALPC is increasing to improve the properties of bone cement and its antimicrobial performance. The field of nanotechnology based antimicrobial medicine and its application in ALBC is still in its infancy, and not well researched as the case in nano-cancer medicine and cardiovascular drug targeting. However, nanotechnology shows promising results in improving antibiotic release and the antimicrobial properties of PMMA bone cement, while preserving its other characteristics needed for physiological performance. The ideal ALBC should sustain the release of antibiotic or antimicrobial agent at high concentration for > 30 days to prevent early and delayed onset postsurgical infections. At the same time, the addition of the antimicrobial species should not compromise the mechanical properties of bone cement, and it's cytocompatibility with the surrounding tissue. The incorporation of gentamicin loaded MSN in the bone cement is a clear example of how a nanotechnology-based approach improved the release kinetics of gentamicin to reach extended release for 80 days, whilst preserving the mechanical properties and cytocompatibility of the bone cement [143].

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Tables

Table 1. Composition of some commercially available PMMA bone cements.

Constituent*	Cemex® XL Genta LV	Endurance™ Gentamicin	Copal®	Palacos® R+G	SmartSet® GHV
Liquid					
MMA	98.20	98.00	97.98	97.98	97.50
N,N dimethyl-p-toluidine (DMPT)	1.80	≤ 2.00	2.02	2.02	≤ 2.50
Hydroquinone	75	75	75	75	75
Chlorophyll	-	-	0.002	0.002	-
Powder					
Poly(methyl methacrylate) (PMMA)	82.78	65.28	-	-	-
Methyl methacrylate (MMA)/styrene co-polymer	-	18.65	-	-	-
PMMA/MMA co-polymer	-	-	82.65	82.15	80.46
Benzoyl peroxide (BPO)	3.00	1.85	0.75	0.78	0.96
Barium sulfate	10.00	10.00	-	-	-
Zirconium dioxide	-	-	10.03	15.01	14.37
Gentamicin sulfate	4.22	4.22	3.76	2.06	4.22
Clindamycin hydrochloride	-	-	2.82	-	-
Chlorophyll		-	0.002	0.002	-

*The amount of each constituent of a cement is in wt. /wt. %, except for hydroquinone, which is ppm.

Table 2. Mechanical properties for some bioactive cements, PMMA and human bone (cortical and cancellous bone).

		Strength (MPa)		Young's modulus (GPa)	Fracture toughness, K_{IC} ($MPam^{1/2}$)	Reference
		Compressive	Bending			
Bioglass® (45S5)		-	42	35	-	[73]
glass polyalkenoate		175.21	-	12.82	0.63	[169]
Hydroxyapatite		39-103	-	4.5-9	0.15-0.5	[170]
Apatite/wollastonite		1080	220	118	2.0	[73]
PMMA		73-117	50-125	2.552	1.03-2.32	[134,171]
Human bone	Cancellous	2-12	-	0.05-0.5	-	[73]
	Cortical	100-230	50-150	7-30	2-12	[73]

Table 3. Antibiotic combinations used for the impregnation of PMMA bone cement spacers for hip and knee prosthetic infections.

Reference	Antibiotic combination used per 40g PMMA
[172]	0.76 g gentamicin + 1 g vancomycin
[173,174]	0.25 g gentamicin + 2 g vancomycin
[175]	1.2-4.8 g tobramycin + 1-2 g vancomycin
[176]	4 g vancomycin + 2 g piperacillin
[177–179]	3.6-4.8 g tobramycin + 4 g vancomycin
[180]	4.5 g piperacillin-tazobactam + 2 g vancomycin + 1 g erythromycin

Table 4. Some of the commercially available antibiotic loaded bone cement brands.

Brand	Antibiotics	Antibiotic used per 40g PMMA	Manufacturer
Palacos R+G	Gentamicin	1.0 g	Zimmer
Palacos LV+G	Gentamicin	1.0 g	Zimmer
CMW 1	Gentamicin	1.0 g	DePuy
CMW 2	Gentamicin	1.0 g	DePuy
SmartSet GHV	Gentamicin	1.0 g	DePuy
SmartSet GMV	Gentamicin	1.0 g	DePuy
Simplex P	Tobramycin	1.0 g	Stryker
Copal G+V	Gentamicin + Vancomycin	0.5 g + 2.0 g	Heraeus
Copal G+V	Gentamicin + Clindamycin	1.0 g + 1.0 g	Heraeus

Table 5. Summary of mechanical properties for different PMMA nanocomposites.

	Compressive strength (MPa)	Bending strength (MPa)	Bending Modulus (MPa)	Fracture Toughness ($MPam^{1/2}$)	Vickers Hardness (MPa)
Liposomes (L31)	80.8	79	3200	3.0	26.6
Mesoporous silica	85	-	2100	-	-
Carbon nano-tubes	8.7	-	-	-	-
Hydroxyapatite nano-rods	43.5	-	-	-	-
Clay nano-tubes	-	35	-	-	-

Table 6. Summary list of nanotechnology based antibiotic loaded PMMA bone cements.

Nano-carrier	% of NPs in bone ce-ment	Loading capacity of gentamicin	Dura-tion of release	% of gentami-cin released	Test-ed bacte-ria	Limitation	Referen-ce
Liposomes		----	30 days	22	S. aureus	--	[142]
Mesoporous silica	10	----	80 days	60	---	--	[143]
Carbon nano-tubes	5	----	60 days	75	----	Cytotoxici-ty, negative impact on mechani-cal properties	[143]
Hydroxyapatit e nano-rods	32	----	60 days	75	----	Negative impact on mechani-cal properties	[143]
Clay nano-tubes	5-7	10-15	10 days	60	S. aureus , E. coli	Burst release	[144]

Table 7. Summary list of nanotechnology non-antibiotic based antimicrobial PMMA bone cements.

Type of Antimic-robial nanopar-ticles	% of NPs in bo-ne ce-me nt	Dura -tion of rele a-se	% of antimic-robial released	Antimic-robial spectrum	Tes-ted bacte-ria	Mode of action	Limitations	Referen-ce
Chitosan	15	---	---	Broad spectrum (Gram positive and Gram negatibe)	S. aureus, S. epider midis	Interacti on with negativel y charged cell wall and cell lysis.		[150]
QCS	15	---	---	Broad spectrum (Gram positive and Gram negatibe)	S. aureus, S. epider midis	Interacti on with negativel y charged cell wall and cell lysis.		[150]
dendrime r	10	30 days	---	Broad spectrum (Gram positive and Gram	S. aureus, E coli, P. aerugi	Interacti on with negativel y charged	Cytocompa tibility problems	[149]

				negatibe)	nosa	cell wall and cell lysis.		
Silver nanoparticles	1	28 days		Broad spectrum (Gram positive and Gram negatibe)	P. aeruginosa, A. baumannii, S. aureus, P. mirabilis	Ag NPs or Ag ions can interact with DNA replication, respiratory chain and cell division.	Negative effect on mechanical properties	[155]
oleic acid capped Silver nanoparticles	0.05			Broad spectrum (Gram positive and Gram negatibe)	S.aurbeus MRSA S. epidermidis A. baumannii	Ag NPs or Ag ions can interact with DNA replication, respiratory chain and cell division.		[156]
Tiopronin capped Silver nanoparticles	0.1			Broad spectrum (Gram positive and Gram negatibe)	MRSA	Ag NPs or Ag ions can interact with DNA replicatio		[157]

						n, respiratory chain and cell division.		
Propyl paraben	7	5		Broad spectrum antibacterial (Gram positive and Gram negatib) and antifungal activity	S.aureus MRSA S. epidermidis A. baumannii	Inhibition of the synthesis DNA and RNA or ATPases and phosphotransferases		[181]

Table 8. Nano-formulations with potential use in bone cements.

Nano-carrier system	Antimicrobial loaded	Duration of release	Loading efficiency	Method of preparation	Application	Reference
LbL coated Mesoporous silica	Gentamicin	10 Days	211 µg/mg		Promising for future applications to coat biomedical device surfaces such as pacemakers	[162]

					and other implanted devices.	
Au	Gentamicin	7 days	38μg/ml			[165]
Ca-silicate	Chlorhexidine	9 days	---		intra-canal medication in dentistry or a new bone defect filling material for infected bone defects.	[166]
PLGA	Gentamicin	16 days	22 μg/ml	Emulsion evaporation	Treating sepsis and Pseudomonas infections	[167]
Hydroxyapatitie nano-rods	Gallium nanoparticles	---	16%	ultrasonic emulsification	tissue engineering, wound healing, bone fracture repair, prevention of infections during implantation	[168]

Figure captions

Figure 1 Total joint replacements undertaken during 2014: (a) Hip and (b) Knee prosthesis, adapted from National Joint Registry (NJR 2015).

Figure 2 Total hip replacement: (a) cemented implant, (b) cementless implant.

Figure 3 Cemented total hip replacement (functions of bone cement).

Figure 4 The number of TKR procedures performed in the UK between the years 2003-2014, adapted from NJR, 2015.

Figure 5 The number of THR in UK between the years 2003-2014, adapted from NJR, 2015.

Figure 6 Free radical polymerization reaction of PMMA .

Figure 7 Proposed liposome-Pluronics structure.

Figure 8 Scheme of (a) GTMC mixed with bone cement (b) bone cement formulated with MSN at low loading and (c) effective diffusion network formed by MSN in PMMA based bone

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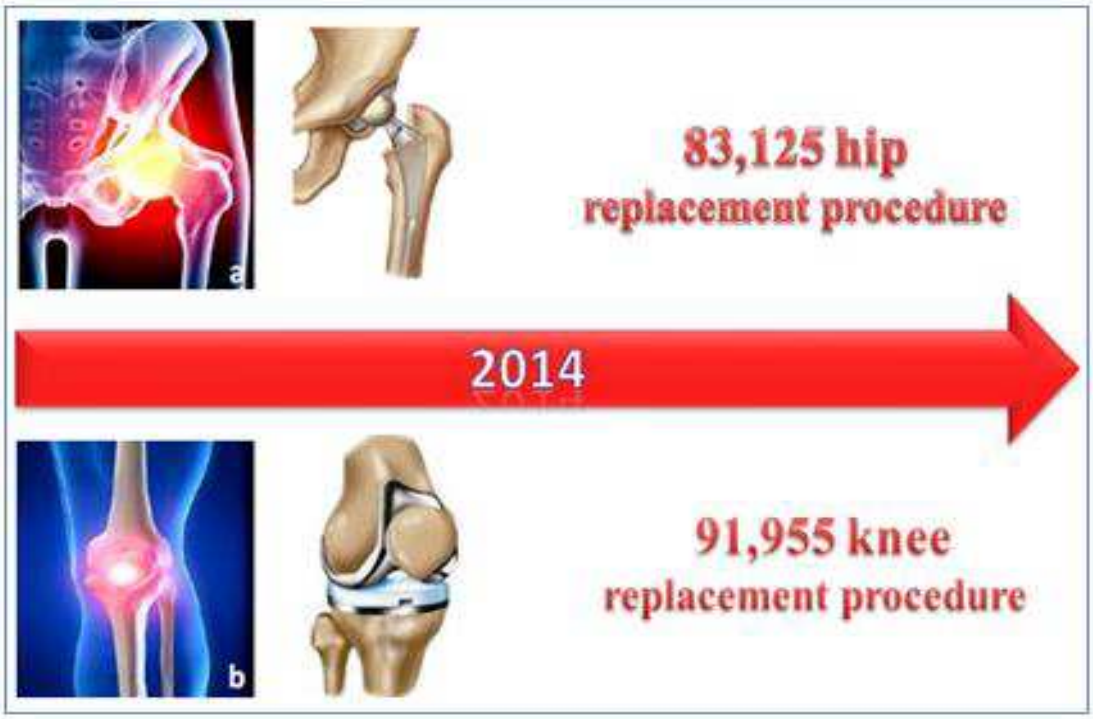
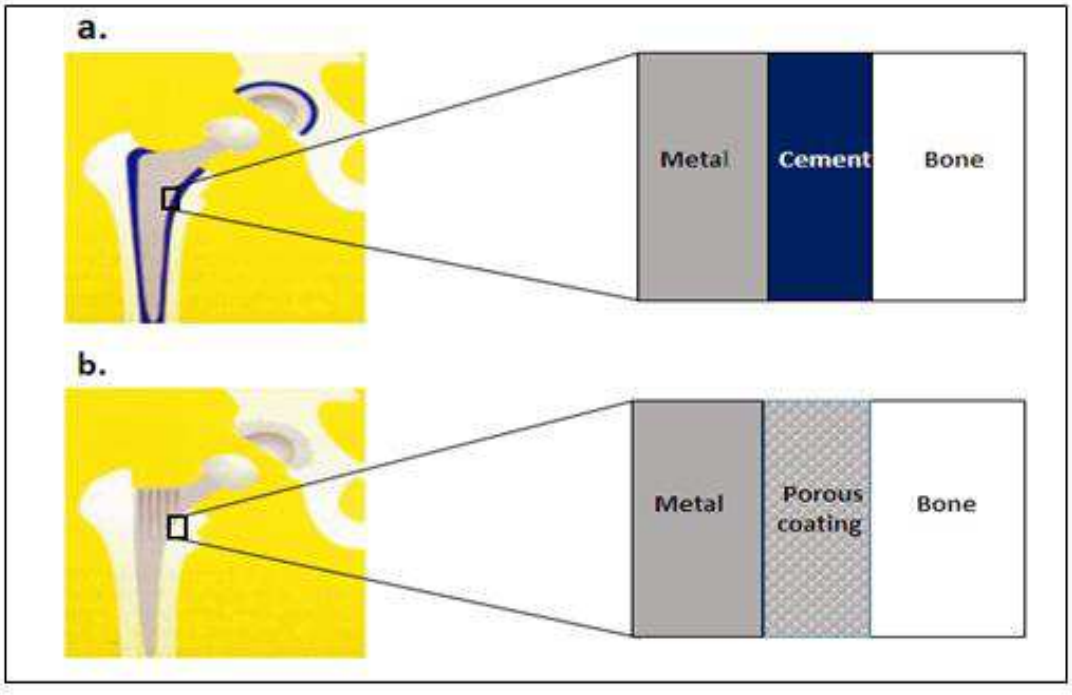


Figure 1



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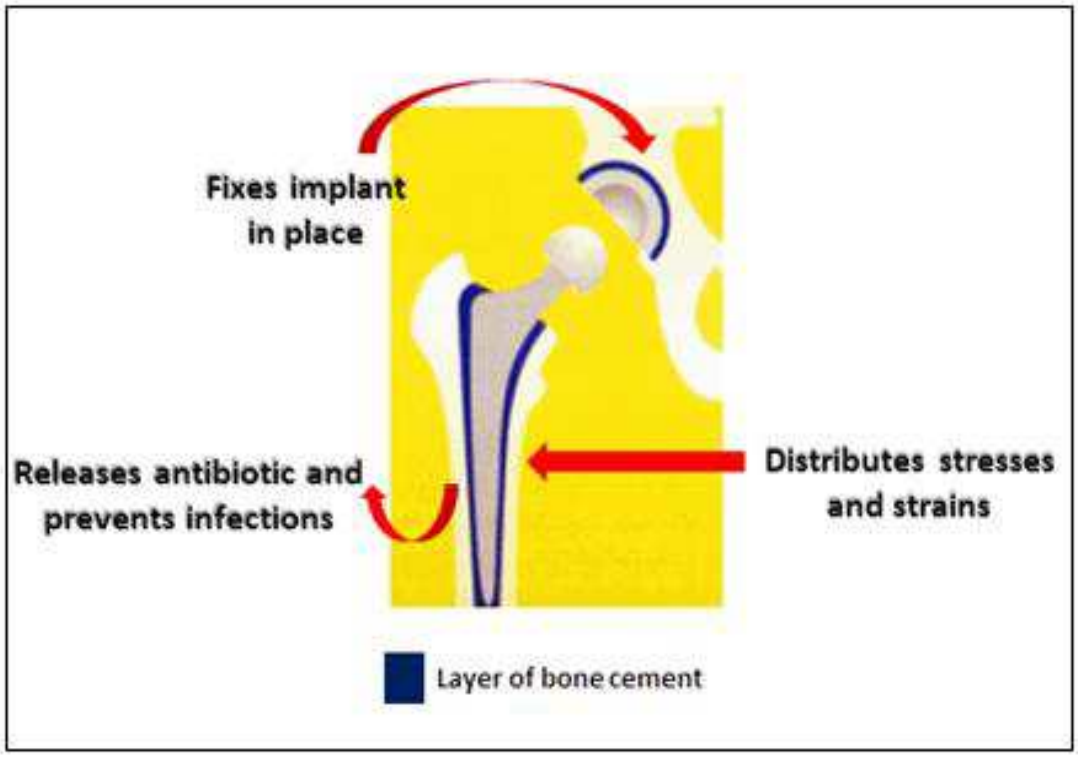

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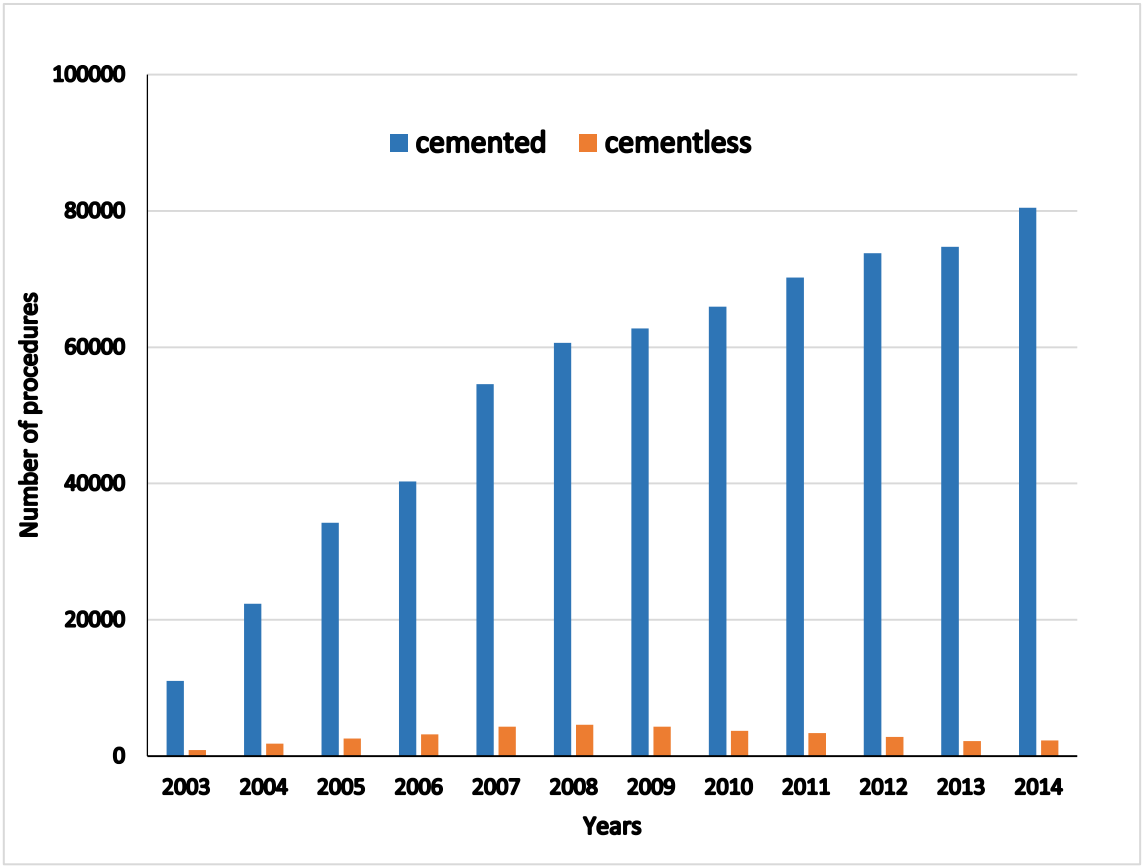

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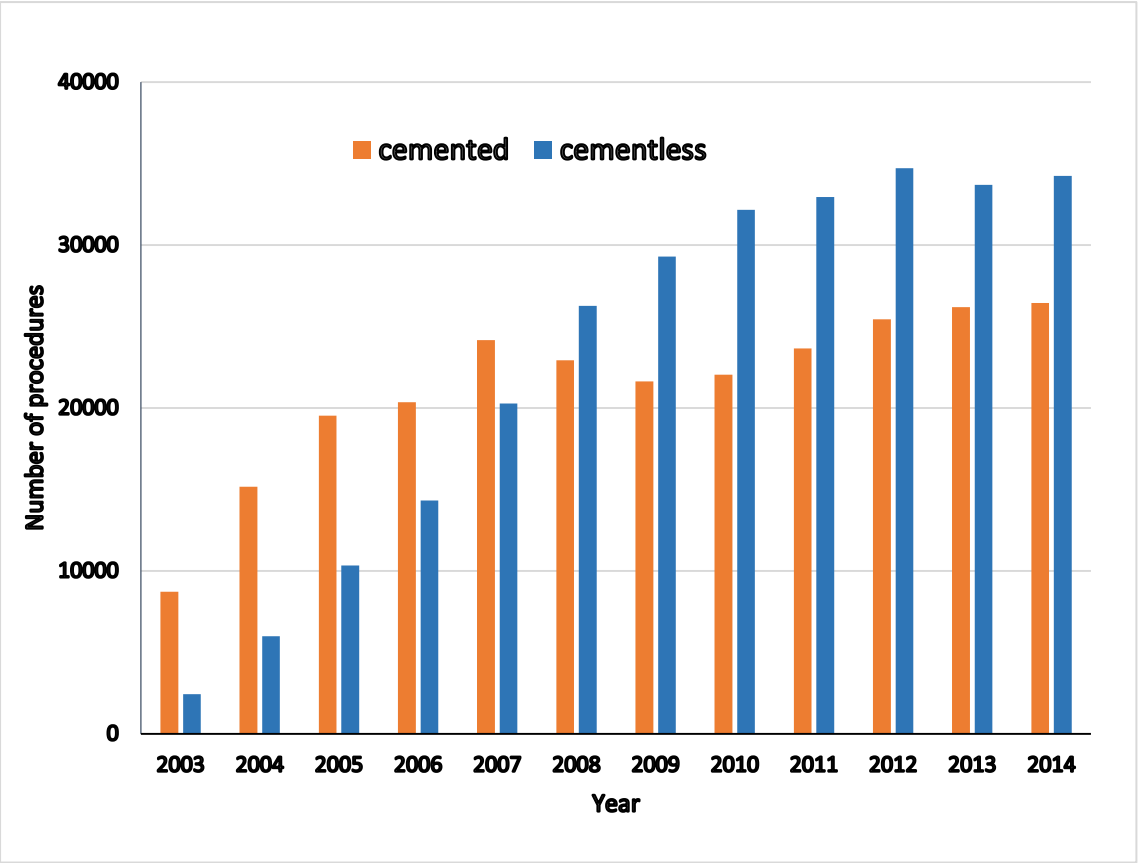

Figure 5

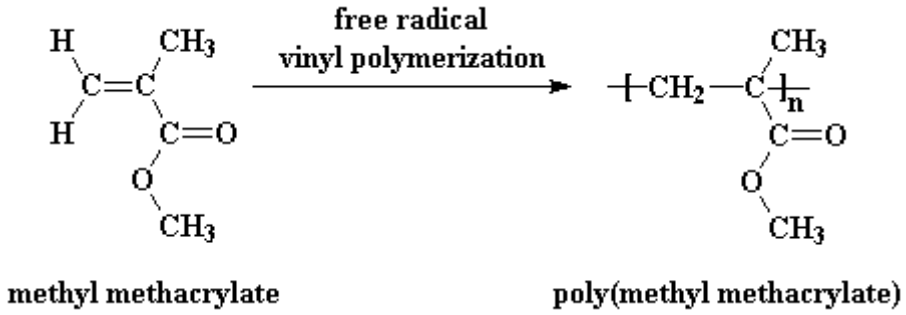

Figure 6

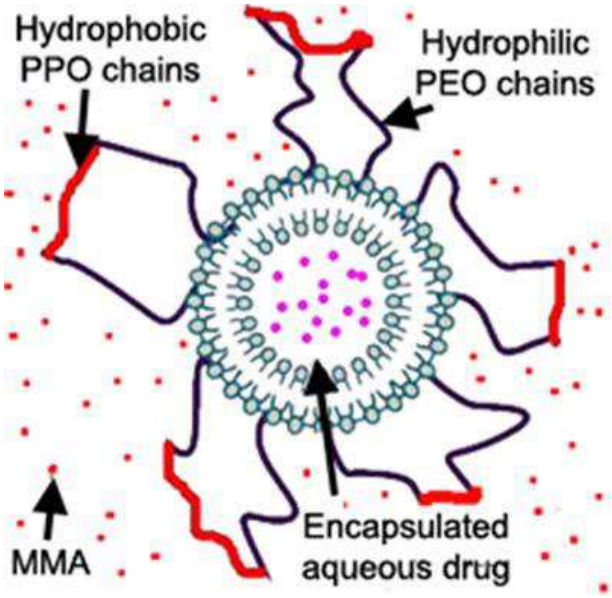

Figure 7

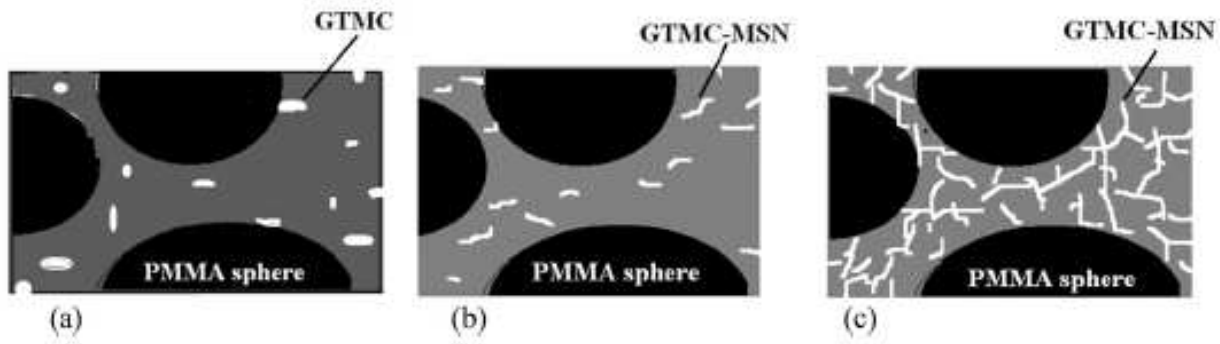

Figure 8