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REVIEW ARTICLE

Engineering the bone reconstruction surgery: the case of the masquelet-induced membrane technique

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Abstract

The reconstruction of large bone defects remains challenging for orthopedic surgeons. Autologous bone grafts (ABGs) are the gold standard treatment for limited size defects, but larger bone defects (>5 cm) require the use of more sophisticated techniques, such as the Masquelet technique. Over the last three decades, the Masquelet or induced membrane technique (IMT) has become increasingly popular as it does not require high-precision microsurgery skills and the time taken to achieve bone consolidation is independent of the length of the defect. IMT is a two-stage procedure. In the first stage, a polymethylmethacrylate (PMMA) cement spacer is implanted into the bone lesion and a physiological immune reaction initiates the formation of a fibrotic induced membrane (IM) with both angiogenic and osteogenic properties. The second stage, performed several weeks later, involves removal of the spacer followed by the implantation of a standard ABG in the preserved IM cavity for subsequent bone repair. In this extensive review, we explain how the success of this surgical procedure can be attributed to the synergy of four key components: the inducer (the PMMA cement), the recipient (the IM), the effector (the bone graft) and the modulator (the mechanical environment). Conversely, we then explain how each key component can contribute to the failure of such treatment. Finally, we discuss existing or emerging innovative and biotechnology-oriented strategies for optimizing surgical outcome with respect to the four components of IMT described above.

Keywords Masquelet · Induced membrane · Orthopedic surgery · Bone repair · Foreign body reaction

Introduction

Bone has a high innate capacity for regeneration but, in many clinical conditions, spontaneous bone-healing mechanisms are undermined. Typically, following extensive diaphyseal bone loss caused by bone tumor resection, congenital malformations or high-energy trauma (road accidents, sports accidents, gunshot wounds and war injuries due to explosive devices) the bone fragments may fail to unite without surgical intervention.

The orthopedic surgeon Frederick Albee was the first to perform bone grafting at a non-union site in 1914. For more than a century, autologous bone grafts (ABGs) have remained the standard of care for the treatment of relatively small bone defects with adequate soft-tissue coverage. Indeed, more than 2.2 million bone-grafting procedures are performed annually worldwide, making bone grafting the second most frequent type of tissue transplantation after blood transfusion [1]. The use of ABG has the advantage that the implanted material consists of the patient's own

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tissues, decreasing the risks of graft rejection and disease transmission. However, the autograft technique has several disadvantages related to the harvesting process, donor site morbidity and pain, as well as the limited volume of the material available. Furthermore, bone autograft techniques are not recommended for lesions of more than 5 cm in length, due to rapid graft resorption.

The reconstruction of large defects (>5 cm) of diaphyseal bones was revolutionized by a new surgical approach introduced by Professor Alain-Charles Masquelet in 1986. A key characteristic of this technique, popularized as "Masquelet's induced membrane technique (IMT)" at the turn of the century, is the preparation of the bed graft through the creation of a biologically privileged membrane at the site of the defect. During a first stage, a polymethylmethacrylate (PMMA) spacer is molded to fill the bone defect. The implantation of this spacer initiates a physiological immune reaction known as the "foreign body reaction" (FBR), leading to the formation of a fibrotic encapsulation membrane known as the induced membrane (IM), which surrounds the spacer. A second surgical intervention is generally performed six to eight weeks later and involves the removal of the spacer with maximal preservation of IM integrity. A standard ABG is then implanted in the IM cavity to mediate subsequent bone repair.

IMT has at least two major advantages. First, no sophisticated equipment or close surgical monitoring are required, and the time required for bone consolidation is independent of defect length. Second, IMT is, technically, a 'simple' surgical approach that does not require microsurgery skills.

These advantages have driven an increase in the use of IMT over the years. The number of studies of IMT has also increased over time. A PubMed database search with the keywords "induced membrane technique" and "Masquelet technique" identifies about 300 publications, 90% of which were published in the last 10 years.

Origin, description and mechanism of action of IMT

The induced membrane is generated by a foreign body reaction

In the early 2000s the IM was described as either a periosteum-like or a synovial-like membrane, according to its osteogenic properties and histological characteristics, respectively [2]. However, it rapidly became clear that a foreign body reaction (FBR) was involved in its formation. Indeed, all surgically implanted biomaterials induce FBRs of various intensities. Regardless of the body site at which implantation occurs, this non-specific immune response consists of five different steps as seen on Fig. 1 [3–5]:

(1) In the minutes immediately following implantation, plasma proteins are adsorbed onto the surface of the biomaterial, leading to the formation of a provisional matrix;
(2) Between one and three days after implantation, neutrophils are recruited to the biomaterial surface, where they initiate an acute inflammatory response;
(3) Later (from about 3 to 10 days post-implantation), a chronic inflammation response occurs, with the infiltration of lymphocytes and monocytes, followed by the differentiation of monocytes into macrophages;
(4) Foreign body giant cells (FBGCs) then form through macrophage activation and fusion, and these FBGCs resorb the foreign biomaterial through phagocytosis;
(5) Finally, a sustained recruitment and activation of fibroblasts is observed resulting in the synthesis of a collagen-based extracellular matrix (ECM) that forms a fibrous capsule surrounding the foreign body.

Thus, the FBR resorbs the biomaterial and attempts to isolate it from the rest of the tissue implanted. In physiological terms, the IM in the Masquelet technique is, thus, a fibrotic encapsulation membrane generated to surround the PMMA spacer in the context of bone damage. In this sense, the IM displays typical features of FBR membranes. The IM consists of a collagen-based matrix. It is about 1.6 mm thick in humans, and up to 1.0 mm thick in other mammals used as models. Histologically, the IM has two or three distinct regions (Fig. 2), as demonstrated in humans [2, 6, 7] and in mammalian models [8–11].

Besides the typical histological features of a foreign body fibrous capsule, the IM also has both specific angiogenic and osteogenic properties. Several immunochemistry investigations in animal models or humans have shown that the IM secretes various cytokines and growth factors beneficial to tissue and bone repair, such as BMP-2, VEGF, vWF, Ang-2, TGF-β; FGF-2 and PGE-2 [2, 10–15]. Moreover, IMs from both humans and animal models have been shown to contain bone marrow stem cells (BMSCs) able to differentiate into osteoprogenitors contributing to bone regeneration [2, 7, 16]. The human IM encloses many CD31-positive epithelial cells in addition to CD146-positive pericytes, providing evidence that the IM has a strong angiogenic potential, thus enhancing the active formation and maturation of blood vessels [2].

The osteogenic and angiogenic properties of the IM are maximal in animal models after an induction time of four weeks, as demonstrated by Tacharla et al. [17]. Bidirectional exchanges occur between the IM and the damaged

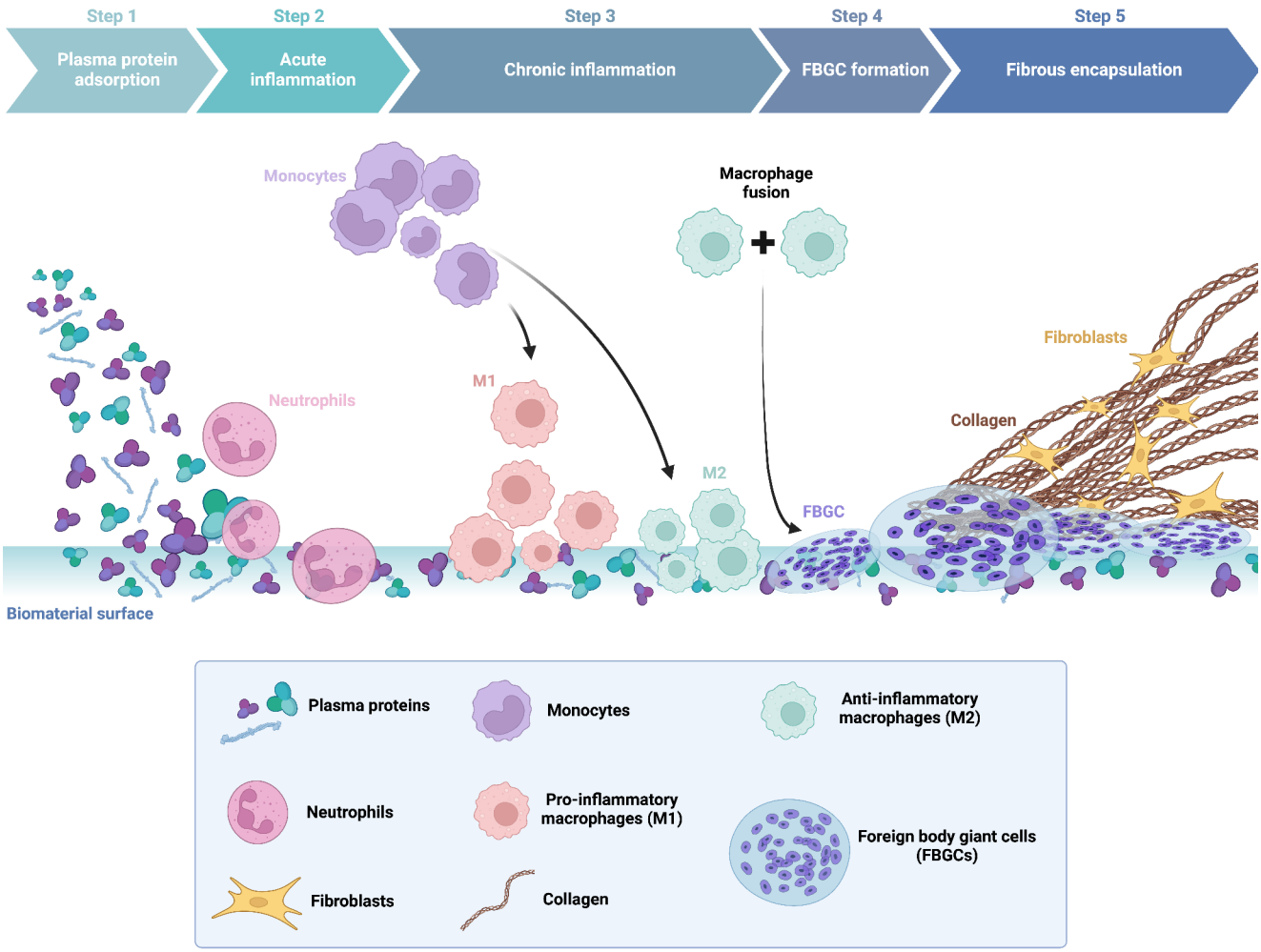

Fig. 1 The foreign-body reaction underlying induced-membrane formation. After PMMA spacer implantation, plasma protein adsorption occurs rapidly at the cement surface (step 1), triggering neutrophil mobilization, which initiates an acute inflammatory response (step 2). An infiltration of lymphocytes and monocytes then occurs, followed by monocyte differentiation into macrophages over a period of several days, corresponding to the chronic inflammation response (step 3). In the next step (step 4), macrophages are activated and fused into foreign body giant cells (FBGC). The formation of these cells is a physiological attempt to eliminate the PMMA cement through phagocytosis. Finally (step 5), fibroblasts are recruited and activated to produce a collagen-based extracellular matrix (ECM) involved in the formation of a fibrous capsule to isolate the PMMA cement from the patient's tissues. Created with BioRender.com

bone site environment, resembling a mutualistic symbiosis in the living world. Following the first surgical intervention, the IM is induced and acquires osteogenic and angiogenic properties from the immune and injured environment. After the second surgical intervention, the IM cavity creates a beneficial microenvironment, acting as an endogenous biological incubator and enhancing bone graft implantation and the subsequent bone-healing process. Ultimately, IMT is a conceptually unique technique involving the interaction between a spacer (the inducer), an IM (the recipient) and a graft (the effector), all of which are influenced by the mechanical environment as a modulator (Fig. 3).

Each of these components can be studied from many different angles, in diverse research domains including immunology, cell biology, biomechanics, biomaterial science, bone tissue engineering and guided bone regeneration. In this review, we will first look at the reasons for clinical failure. We will then consider likely future developments and advances in biotechnology that may make it possible to optimize the efficacy of this technique.

IMT failures

Despite its increasingly high rates of successful IMT use worldwide and the efficiency with which this technique promotes bone repair, IMT failures have been reported in several prospective and retrospective studies or database reviews. Reported rates of IMT failure in adults are generally between 11% and 18% but they can be decreased to

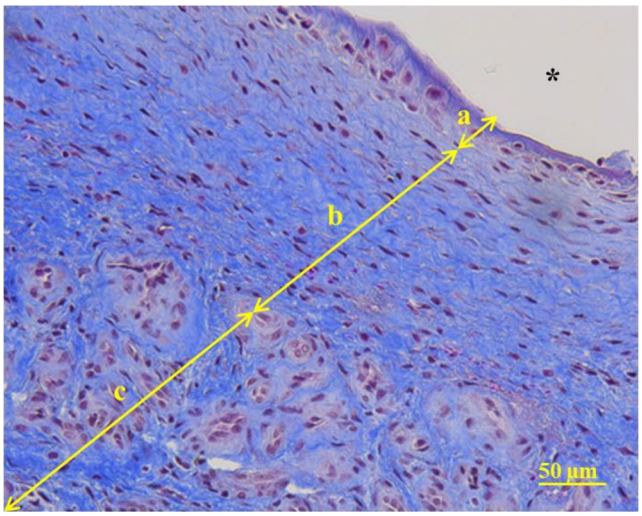

Fig. 2 Representative organization of a human induced membrane stained with Masson-Goldner trichrome dye. The black asterisk outside the section indicates the location of the PMMA spacer responsible for membrane induction. Three different layers are identified in the induced membrane (indicated by yellow letters). a: is the "inner" layer closest to the spacer consisting principally of monocyte-derived cells. b: is the "intermediate" layer containing small blood vessels, fibroblast-like cells and collagen fibers orientated in parallel to the PMMA spacer. c: is the "external" layer characterized by unorganized collagen fibers, a combination of myofibroblasts, fibroblast-like cells and large blood vessels. The scale bar represents 50 µm

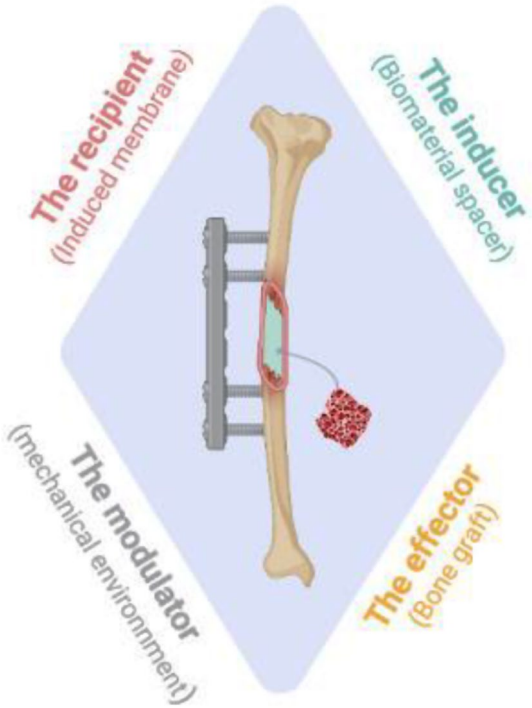

Fig. 3 The concept underlying the induced-membrane technique. The success of the procedure depends on the interaction between an inducer (the spacer), a recipient (the IM) and an effector (the graft). The mechanical environment of the fracture acts as a modulator of these components. Created with BioRender.com

below 10% (as low as 7.6%) by revision surgery to treat complications [18–22].

Several studies have investigated the reasons for IMT failure. We previously proposed a scheme for classifying IMT failures according to whether they were preventable or unpreventable [23]. Most IMT failures are preventable and result from an inappropriate assessment of the clinical situation or a lack of technical expertise and know-how for the surgery required. Most of the remaining, non-preventable failures are related to biological deficiencies of the IM either from physiological origin or due to precise circumstances.

Whether preventable or unpreventable, failures involve all four components of the IMT (the inducer, recipient, effector and modulator) as previously defined (Fig. 4).

Inducer-related risk factors for IMT failure

PMMA is currently the only biomaterial clinically validated worldwide for IM induction via Masquelet's technique. PMMA (Plexiglas) synthesis was patented in 1933 and this material was first used as bone cement in the early 1940s. For medical applications, PMMA cement is obtained by mixing ground PMMA powder and liquid methylmethacrylate (MMA) monomers in the presence of two co-initiators: N,N-dimethyl-p-toluidine (DmpT) and benzoyl peroxide [24]. The resulting dough-like material produced by the chemical reaction between these components has the advantage of being easily molded by hand and modeled into the bone lesion before its final polymerization. Moreover, it can be loaded with heat-resistant antibiotics. However, these advantages of PMMA cement are also major limitations. The positioning of the cement spacer appears simple but can affect the final outcome of surgery. According to the first description of this technique, the PMMA should be implanted as a block cylinder with, importantly, the edges of the bone wrapped in 2–3 cm of cement [25]. However, PMMA blocks can be difficult to remove, especially in cases of complex bone geometry (tibial pilon fractures, for example). Attempts have been made to facilitate the removal of the cement spacer by filling the defect with flat "pebbles" or beads of PMMA. The resulting PMMA spacers are heterogeneous in shape, which may decrease the reproducibility of the biological properties of the IM between patients. Moreover, PMMA beads and pebbles generate irregular IMs that are not ideal for graft containment [26]. It has been confirmed in other studies that PMMA blocks are more effective than beads for healing large defects [27].

Another issue regarding PMMA is the exothermic reaction (about + 60 °C) that occurs during polymerization, which may damage bone edges and soft tissues close to the lesion. In addition, the high temperatures reached limit the panel of antibiotics that can be included in the PMMA

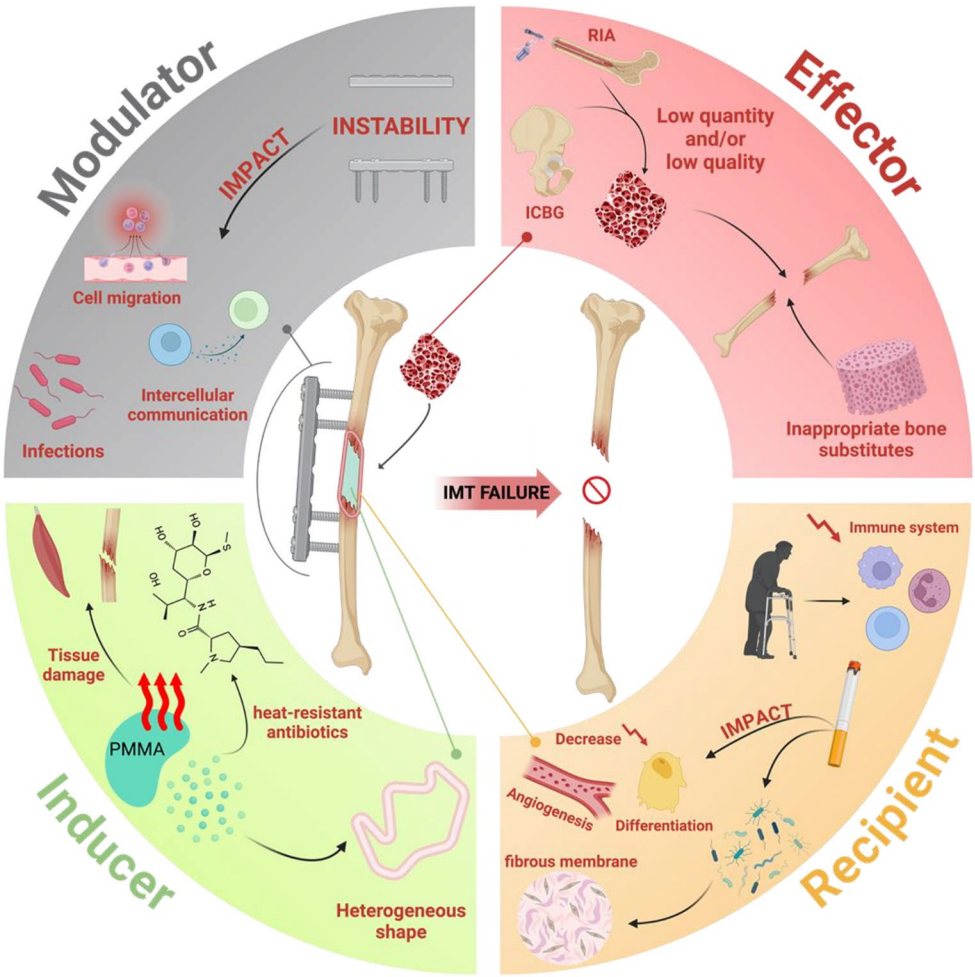

Fig. 4 Overview of the principal factors associated with a poor outcome of IMT. According to the induced-membrane concept illustrated in Fig. 3, various factors may lead to failure of the induced-membrane technique (IMT). Failures related to the "inducer" component generally result from several drawbacks of the PMMA spacer itself, such as the heat generated by PMMA cement polymerization. The "recipient" component may be adversely affected by an unhealthy patient lifestyle, such as smoking, or old age. For the "effector" component, a small amount of autologous bone graft material or the poor quality of the graft may decrease bone repair efficiency. The use of an inappropriate bone substitute may also have a similar effect. Finally, the instability of the osteosynthesis system (bone fixators) provides an example of the contribution of the "modulator" component to IMT failure. Created with BioRender.com

to heat-resistant antibiotics only. The antibiotics most frequently included in PMMA for IMT are glycopeptides and aminoglycosides (gentamicin, vancomycin), generally at concentrations of 1–5% w/w in commercial brands of PMMA [28]. In clinical practice, it is not uncommon for surgeons to add additional antibiotics, including vancomycin or tobramycin, to the PMMA directly before its polymerization [29]. The addition of antibiotics to the spacer also rapidly raised another concern regarding the impact of antibiotics on the quality of the IM and bone healing. Xie et al. [30] performed IMT in a rabbit model involving the use of PMMA spacers loaded with vancomycin at concentrations of 2.5–25% (w/w). The authors showed that only relatively high concentrations of vancomycin (15–25% w/w) have negative effects on the proliferative, osteogenic and angiogenic capacities of IM. Similarly, spacers loaded with lower vancomycin concentrations (2.5–10% w/w) did not interfere with new bone formation, whereas higher concentrations have a significant detrimental impact on bone repair.

In conclusion, PMMA spacers loaded with high concentrations of antibiotics seem to have a deleterious effect on the osteogenic properties of the IM, resulting in more detrimental effects on subsequent bone regeneration in animal IMT models. Following the implantation of such spacers in vivo, there is a rapid burst of antibiotic release that is thought to be toxic, with a detrimental impact on IM maturation. The benefits of PMMA supplementation with antibiotics are well-established in septic conditions, but surgeons should be aware that the local and uncontrolled delivery of antibiotics is not entirely without risk in the context of IMT.

Recipient-related risk factors for IMT failure

Recipient-related risk factors for IMT failure include a defective IM bed or bone environment due to various patient-independent factors. For example, a lack of control of infection in the IM bed is the main preventable risk factor for IMT failure as it can account for up to 68% of complications [18, 31]. *Staphylococcus aureus* and *Staphylococcus epidermidis* together account for more than 50% of infections during IMT procedures [18, 22].

In a rat IMT model at the first surgical stage [32], *Staphylococcus aureus* infection appears to trigger the formation of a thicker fibrous capsule surrounding the PMMA-derived

spacer than that observed in the absence of infection. The IM generated in infected conditions displays inflammatory cell infiltration and an upregulation of mRNAs for the pro-inflammatory cytokines IL-1β, IL-6, and TNFα and the anti-inflammatory cytokine IL-10 mRNAs, together with a downregulation of the BMP-5 mRNA relative to uninfected conditions. According to the authors, the persistent inflammation induced by the infection (increase in IL-1β, IL-6 and TNFα levels) results in a less osteogenic IM, as demonstrated by the low levels of mRNA for the osteogenic factor BMP-5.

Smoking has also been identified as a risk factor for complications in IMT procedures. Nicotine alters both blood perfusion in soft tissues and cytokine gene expression related to osteoblast differentiation and neovascularization in bone, potentially accounting for the negative impact of smoking in patients undergoing IMT. Patient age is also a significant risk factor for delayed bone union following IMT treatment [33].

Another clinically relevant risk factor for IMT failure that has been little studied is changes in the patient's immune system. The membrane is a foreign body–induced granulation tissue. As such, any immune system deficiency related to anti-inflammatory drug medication or immunosuppressive treatment is likely to interfere with the formation and biological properties of the IM [34]. Surprisingly, very few studies on this topic have been published. In 2018, Sagardoy et al. investigated the properties of IMs collected from the hindlimbs of rats undergoing an external beam radiotherapy protocol [35]. A 6-mm bone defect filled with PMMA was created in the hindlimbs of the rats. The irradiation protocol was identical to that used in humans as an adjuvant therapy for head and neck squamous cell carcinomas. It consisted of a total dose of 50 Gy and was initiated three weeks after spacer insertion. Sagardoy et al. reported that irradiated IM were significantly thicker, with a more extensive inflammatory cell infiltrate [35]. In addition, a significant decrease in the density of blood vessels within the IM was observed after irradiation. The authors suggested that the deleterious effects of irradiation on the IM might be related to an irradiation-induced alteration of PMMA leading to the release of toxic degradation products. However, even if PMMA degradation is a major factor contributing to the changes in the IM, it is not the only one, given the significant immunosuppressive effects of irradiation [36]. Our understanding of the contribution of immune system modulation to IMT failure remains incomplete.

Effector-related risk factors for IMT failure

Effector-related failures result from the grafting of an inadequate material into the the IM during the second surgical intervention. The gold standard source of graft material for standard IMT is an iliac crest bone graft (IC-BG). However, the volume of bone that can be harvested from the iliac crests is limited (about 25 to 30 cm^3 per crest). This may be insufficient if iterative bone grafting is required, given that the mean volume of ABG required is 7 cm^3 per cm of defect for a diaphyseal lesion of human femur for example [37]. In such situations, clinicians may choose to use alternative graft sources or graft expanders such as allograft or xenograft material (Orthoss, Geistlich Pharma) or tricalcium phosphate (TCP). Both the use of expanders and the ideal ratio of autograft to expander are the subject of intense debate within the orthopedic community. Flierl observed that the time to union was significantly shorter for patients treated by IMT with autografts alone than for patients receiving either allografts or a combined allograft/autograft mixture [38]. A more recent study by Lu reached the same conclusion [39]. However, other studies reported no increase in failure rates with bone graft supplementation [40, 41]. Most studies have recommended the use of a bone-graft expander-to-autograft ratio $\leq 1/3$. Several studies have reported good results with a high proportion of bone substitute [40], or even with allograft or bone expanders alone [42, 43] but a percentage of bone-graft expander of more than 40% by volume is generally considered a risk factor for failure.

Mechanical environment-related IMT failure

Mechanical forces underpin both the immune response and the bone-healing process at multiple levels, by regulating cell-surface receptor activation, cell migration, intracellular signaling and intercellular communication [44–46]. The initial description of the IMT procedure recommended the use of an external frame as the fixator at the first surgical intervention and a reinforced definitive fixation at the second stage. Several teams have since demonstrated the versatility of the technique in terms of the choice of fixation method. Many modifications to the original description have been introduced, including the use of a nail or a plate for definitive fixation at both stages of the technique [47–49]. However, there is still no clear consensus on the optimal bone fixation approach for IMT. A preclinical study in rabbits demonstrated that rigid fixation, achieved by combining PMMA cement with plates, at the first surgical intervention enhanced the osteogenic and angiogenic potential of IMs over that of IMs induced with the PMMA spacer alone [30]. In another preclinical study in mice, plate-fixed IMs were found to have a lower capacity for bone growth [50]. In humans, a large retrospective study by Siboni et al. [22] suggested that an absence of rigid fixation during the second stage of treatment might impede healing. Moorwood

et al. demonstrated that, when treating lower-limb defects (in 121 patients), the use of intramedullary nails during the second stage of IMT leads to significantly faster union and a requirement for fewer surgical revisions than the use of plates [51]. However, other researchers investigating the relationship between the effects of patient factors and technique variations on IMT outcomes reported no clear superiority of intramedullary nail use over the use of plates [52]. Mathieu et al. recently proposed a strategic guide recommending the most appropriate osteosynthesis devices for stages 1 and 2 of IMT based on the infection status of the bone lesion (infected or non-infected) and the nature or location of the injured bone [53].

Future developments and biotechnological advances likely to optimize the technique

Over the last decade, several suggestions have been made for the optimization of IMT outcomes. We describe here the principal advances in research, focusing on innovative biotechnological approaches for improving the therapeutic efficiency of IMT or meeting the clinical needs of orthopedic surgeons (See Fig. 5).

Inducer-based strategies

Inducer composition and topography

The biological process leading to generation of the IM is based on the FBR, as described in section "The induced membrane is generated by a foreign body reaction". Macrophages play a crucial role in the FBR by orchestrating the inflammatory environment around the implanted biomaterial. Macrophages are highly plastic cells that can adopt a spectrum of functional phenotypes upon activation, ranging from M1 (pro-inflammatory) to M2 (pro-healing) profiles, depending on the shape and surface properties of the biomaterial (chemistry, porosity, wettability, roughness, stiffness, etc.) [54, 55]. In the context of IMT, bioengineering strategies can modify the PMMA (change in surface topography, chemical modifications, etc.) or replace it with an alternative biomaterial to influence the immune microenvironment in which the FBR occurs. Such approaches intend to alter the osteogenic properties of the IM with the aim of enhancing bone regeneration. The studies performed in this area are summarized in Table 1.

Luangphakdy's pioneering work marked the first attempt to use a textured PMMA spacer in a caprine model of IMT [56]. The authors used a ribbed (about 2-mm deep grooves) spacer that doubled the surface area of the IM. They did not investigate the effects of the textured PMMA on the biological properties of the IM, but they found that the ribbed PMMA spacer did not modify bone repair efficiency relative to a standard smooth PMMA spacer. Two related studies by Gaio et al. and Toth et al. [8, 57] used a roughened PMMA (grooves about 8 µm deep) in a rat model of IMT. Unlike Luangphakdy, Toth demonstrated a significant effect of alterations to PMMA topography on the membrane, potentially disrupting phase 2 bone formation. Specifically, bone union was observed in 60% of rats treated with smooth PMMA spacers, but only 9% of animals treated with rough PMMA spacers. Apart from the different animal models used, the main factor potentially accounting for the conflicting results between the studies of Luangphakdy and Toth is the depth of the grooves created in the spacer, with Luangphakdy using 2 mm grooves and Toth using deeper 8 µm grooves. At the cellular level, the impact of PMMA microstructure appears to outweigh the effect of macrotexture. More recently, Ziroglu tested three modified PMMA cement mixtures in a rat femoral model of IMT: estrogen-impregnated PMMA (E+PMMA), PMMA supplemented with bone chips (BC+PMMA), and hydroxyapatite-coated PMMA (HA) [58]. The most advanced stage of IM differentiation was achieved with estrogen-impregnated cement. The authors concluded that this alternative spacer is promising for use in IMT and could potentially shorten the duration of bone healing. Ziroglu also tested calcium phosphate cement (CPC) in their study. This alternative biomaterial had previously been evaluated by a Chinese research group in a rat model [59]. CPC-induced membranes were found to produce more VEGF, BMP-2 and TGF-β1 than PMMA-IM. In addition, CPC promoted more endochondral ossification at the broken ends of the bone defect than PMMA.

Various other non-cemented spacers with chemical compositions different from PMMA have been investigated, including titanium [57], polyvinyl alcohol [57], silicone [35], polypropylene [60], polycaprolactone [61] and composite materials consisting of PCL/fumarate [62] and nanohydroxyapatite/polyamide [63]. None of the non-cemented alternative spacers produced IMs with biological properties significantly superior to those of the membranes induced by PMMA cements. In the worst case, PVA failed to generate an IM while bioglass putty induced the formation of a poorly organized IM lacking the necessary expansion capacity for the second stage of the technique [64]. At best, spacers made from metakaolin and nanohydroxyapatite/polyamide yielded IMs with slightly better osteogenic and/or angiogenic properties than the IMs induced by PMMA. Silicone, titanium, polypropylene, and polycaprolactone spacers produce IMs with similar properties to PMMA-IMs. Non-cemented spacers do not appear to improve the induced membrane technique (IMT) significantly, but their ease of use may promote the replacement of PMMA

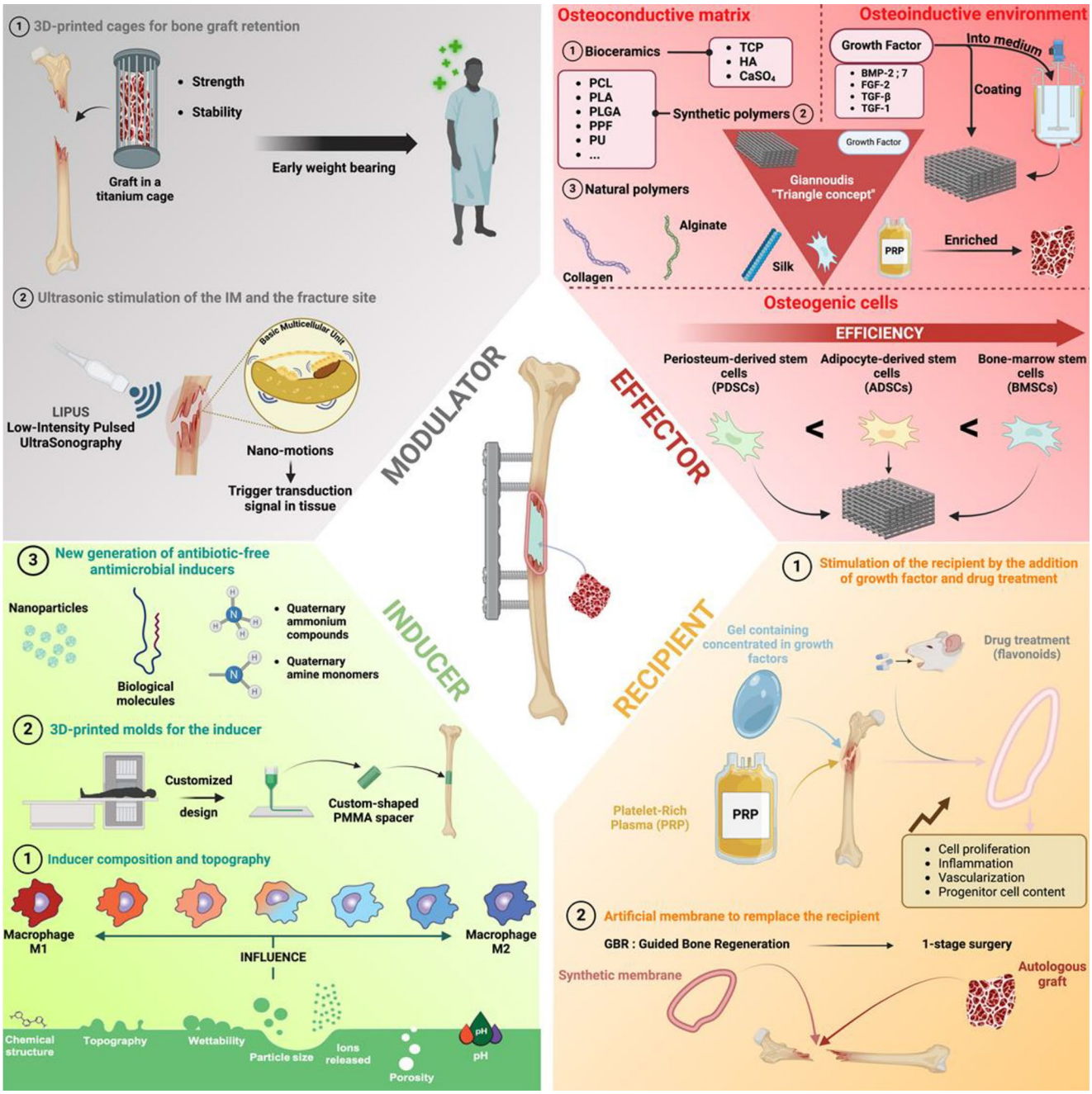

Fig. 5 Overview of promising strategies for improving the therapeutic outcome of the induced-membrane technique. This figure summarizes the most promising medical and technological strategies for increasing the bone-repair efficiency of the induced-membrane technique taking into account the inducer, recipient, effector and modulator aspects of the Fig. 3 IMT concept. Created with BioRender.com

spacers. For example, the use of a polypropylene spacer made from disposable syringes can lead to bone reconstruction if resources are limited and no PMMA is available [65]. Medical-grade silicone is easier to remove than PMMA and may therefore be useful for the treating of complex fractures close to nerves, tendons or blood vessels [35].

Three-dimensional printed molds as inducers

In recent years, attention has increasingly focused on three-dimensional printing technologies in orthopedic surgery because these technologies make it possible for surgeons to create custom-made and anatomically matched implants based on patient characteristics. In the context of IMT, one of the inducer-based strategies for improving surgery

Table 1 Summary of key studies of the effects of inducer modifications on the characteristics of the induced membrane and bone healing outcomes

Authors	Year	Species/bone	Experimental modification of the inducer and groups	Consequence for the IM	Consequence for bone healing
Luangphakdy *et al.*	2017	goat/tibia	Altering surface topography: smooth PMMA vs textured-PMMA	/	No difference in bone formation between textured and smooth PMMA
Ma *et al.*	2017	rat/femur	Altering material: PMMA vs calcium sulfate	In calcium sulfate-IM: higher expression of VEGF, TGF-β1 and BMP-2 + presence of endochondral ossification	In calcium sulfate group, more new bone formation (higher BMD + BV/TV) than in PMMA group
Gaio *et al.*	2018	rat/femur	Altering both material and surface topography: smooth PMMA vs smooth titanium (TI) vs rough PMMA vs rough TI	TI spacers create IMs that inhibit solute transport more. PMMA rough and TI rough -IM shrunk more than IM from smooth spacers.	/
Toth *et al.*	2019			TI-IM are thicker than PMMA-IM but have a similar architecture and expression of BMP2, TGFβ, VEGF, IL6. Roughening increases IL-6 expression.	Bone regeneration is better supported by PMMA-smooth IM (60% union)
Sagardoy et al.	2018	rat/femur	Altering material: PMMA vs silicone	PMMA and silicone spacers create similar IMs.	/
McBride-Gagyi *et al.*	2019	rat/femur	Altering material: PMMA vs titanium (TI) vs polyvinyl alcohol (PVA)	PMMA and TI spacers create similar IMs. PVA fails to create an IM.	PMMA-IM supports functional union, TI and PVA groups fail to achieve the same.
Murison *et al.*	2019	human/metacarpal bone	Altering material: PMMA vs polypropylene (PP) syringe body	PP syringe body creates a membrane macroscopically similar to a cement-related IM	The use of a PP spacer allows to achieve bone union in four critical metacarpal bone defects
Mathieu *et al.*	2021	rat/femur		PMMA and PP syringe body create similar IMs (architectural organization, cell density, BMP2 expression)	PP-IM and PMMA-IM support bone regeneration equally well.
Stahl *et al.*	2021	rat/femur	Altering material: PMMA vs polycaprolactone (PCL) vs MMA-eluting PCL (high-dose PCL-MMA and low-dose PCL-MMA).	No difference in VEGF or BMP-2 expression between IMs induced by PMMA, PCL, high-dose PCL-MMA and low-dose PCL-MMA. Greater vascularization around PCL spacers than around high-dose PCL-MMA spacers.	/
Durand *et al.*	2022	rat/femur	Altering material: PMMA vs Metakaolin (MK)	MK-IMs have an architectural organization similar to that of PMMA-IMs but higher levels of TGF-β and BMP-2.	In the MK group there was a tendency towards higher levels of new bone formation (higher BV/TV).

Table 1 (continued)

Authors	Year	Species/bone	Experimental modification of the inducer and groups	Consequence for the IM	Consequence for bone healing
Wang *et al.*	2024	rat/femur	Altering material: PMMA vs nano-hydroxyapatite/polyamide composite scaffold coated with platelet rich plasma and fibrinogen (PRP-FG-nHA/PA66)	PRP-FG-nHA/PA66-IMs are thinner than PMMA-IM but display higher vessel density.	PRP-FG-nHA/PA66 scaffold decreases the amount of ABG required at the second stage, with smaller amounts bone graft material resulting in healing similar to that achieved with PMMA.
Ziroglu *et al.*	2024	rat/femur	Altering material: PMMA vs estrogen impregnated PMMA (E+PMMA) vs bone chip added PMMA (BC+PMMA) vs hydroxyapatite-coated PMMA (HA) vs calcium phosphate cement (CPC)	Compared to all other groups, E+PMMA and CPC-IM have a better organization and a higher expression of TGF-β and VEGF. The E+PMMA and BC+PMMA groups have higher serum concentrations of bone remodeling markers.The most promising IM was generated with an E+PMMA.	/
Astudillo Potes *et al.*	2024	rat/femur	Altering both material and surface topography: PMMA vs solid polycaprolactone fumarate (PCLF/PCL) vs porous PCLF/PCL.	Both solid and porous PCLF/PCL spacers create collagen-rich IMs similar to PMMA-IMs. Porous PCLF/PCL-IMs have a woven bone formation and higher vessel density than PMMA-IM.	/
Ivaldo Siqueira Silva Junior *et al.*	2024	rabbit/radius	Altering material : F18 bioglass putty	F18 bioglass putty induces IMs with no expansion cavity for the second stage of IMT.	/

involves the use of 3D printing to facilitate the implantation and removal of PMMA cement. Zhang et al. explored the feasibility of fabricating molds with a 3D printer for the production of personalized bone cement [66]. They reconstructed the injured calcaneus in four patients with calcaneal bone defects, based on CT scans of the intact contralateral calcaneus. A mold composed of polylactic acid material shaped to fit the injured calcaneus was then generated with a 3D printer. Finally, PMMA cement was cast in the mold to produce a custom-shaped PMMA spacer for insertion into the calcaneal defect. The IM produced by the shaped PMMA spacer generated in this way closely follows the outline of the calcaneus, thereby enhancing the quality of bone repair following bone grafting [66].

New generation of antibiotic-free antimicrobial inducers

As mentioned above, the use of antibiotic-loaded PMMA to eradicate infection has major disadvantages in the IMT context. Moreover, there is increasing clinical concern about the possible contribution of antibiotic-impregnated bone cements to the development of antibiotic resistance [67]. These concerns have driven attempts to develop new types of PMMA containing no antibiotics, but still having effective antimicrobial activities. A new generation of "antibiotic-free antimicrobial bone cement" (AFAMBC) is emerging. Various strategies, such as the incorporation of nanoparticles into the cement, are being used to confer antibacterial properties on PMMA without the use of antibiotics. Silver is known to have broad antimicrobial effects and PMMA loaded with 1% nanosilver has been shown to have excellent antimicrobial activity without cytotoxicity when tested in vitro against a clinical isolate of Methicillin-Resistant Staphylococcus Epidermitis (MRSE) [68]. Bone cement impregnated with chitosan nanoparticles also has stronger antimicrobial activity against both *S. aureus* and *S. epidermidis* than control plain PMMA [69]. Another kind of AFAMBC consists of bone cement bearing antibacterial motifs derived from a natural biological molecule. For example, Yang [70] recently demonstrated a strong antimicrobial effect in vitro of PMMA into which the antimicrobial peptide ε-poly-L lysine (1.5% w/w) was incorporated. Other inorganic agents, such as quaternary ammonium compounds (QACs), have also been added to PMMA [71]. Deb

et al. described the effect of the inclusion of an antibacterial quaternary amine monomer (QAMA) (quaternized ethylene glycoldimethacrylate piperazine octyl ammonium iodide) into a commercial bone cement [72]. The authors showed that cements prepared with 15% QAMA inhibited *E. coli* growth without toxicity to a human osteosarcoma cell line. The antibacterial functionalization of PMMA is an emerging field, and further in vivo studies are required to assess the safety of this new generation of cements and their ability to reduce infection rates in a clinical context.

Recipient-based strategies

For cases in which biological impairments of the IM bed are suspected, various strategies for improving IM quality before grafting are being investigated. These strategies include coating the IM with growth factors and the systemic administration of medication at stage 1 of the procedure. Efforts are also being made to simplify the procedure and reduce it to a single stage by implanting alternative membranes of synthetic or natural origin, thereby preventing potential dysfunction in the recipient.

Stimulation of the recipient with growth factors

Two Turkish research groups have used animal models to test the effects in IM properties of adding growth factors to the defect. Yilmaz et al. prepared concentrated growth factor (CGF) gel containing osteoinductive growth factors derived from platelets and an osteoconductive fibrin matrix from the venous blood of rabbits [73]. They applied this CGF gel to the bone cement in a rabbit model of IMT. Three and six weeks later, they performed an immunohistochemical evaluation of cell proliferation (Ki-67 staining), inflammation (MAC387 staining), vascularization (CD31 staining) and progenitor cell content (STRO-1 staining) in the IM. The results suggested that the inclusion of CGF gel in the IMT improved the quality of the IM compared to control non-treated IM, by increasing the number of progenitor cells and endothelial cells, together with the proliferation index and inflammation levels. In 2022, the same research group showed that CGF addition supported bone formation by increasing the volume of new bone, as confirmed by micro-CT and histological evaluations [74].

Bilal et al. injected a solution of platelet-rich plasma (PRP) or epidermal growth factor (EGF) into the wound area during PMMA cementing and then performed weekly injections of EGF (25 µg/ml) or PRP (1 ml of fresh in house-prepared PRP) into the bone defect for three consecutive weeks [75]. An analysis of the membrane at six weeks post-surgery revealed that TGF-β, VEGF and CD31 levels were higher in both the PRP and EGF groups than in controls (untreated animals). In addition, treatment with either PRP or EGF resulted in better bone healing assessed radiologically six weeks post-grafting.

Stimulation of the recipient by drug treatment

To our knowledge, only one study has explored the use of pharmaceutical treatment with total flavonoids from *Rhizoma drynariae* (TFRD) or *Plastrum testudinis* (PT) extracts to improve the properties of the IM [76]. Both TFRD and PT are traditional Chinese medicine products with reported anabolic effects on fracture healing through activation of the BMP-Smad signaling pathway and inhibition of NF-κB, respectively [77, 78]. Following oral administration to rats, TFRD and PT increase angiogenesis in the IM by almost 90% and 50%, respectively, four weeks after PMMA insertion. Moreover, the percentage of BMP-2-positive cells in both the TFRD and PT groups was higher than that in control animals. The beneficial effects of the Chinese medicine products on stage 1 of IMT are associated with better bone healing at stage 2.

Use of artificial membranes to replace the recipient

It would be useful to have an off-the-shelf biomimetic membrane of synthetic or natural origin available for use in cases of a defective IM bed. Moreover, the use of an alternative biomimetic IM would eliminate the need for the first surgical step, thereby shortening the overall IMT process. The development of such a one-step strategy is inspired by the guided bone regeneration (GBR) approach commonly used in dentistry.

Dahlin and coworkers introduced the guided bone regeneration (GBR) concept through their work on rats published in 1988. They created bilateral bone defects of standard size in the jaws of the animals. The defect on one side of the jaw was covered with Teflon membranes, whereas the contralateral defect was left uncovered as a control. Six weeks after creation of the defect, complete bone healing was observed on the Teflon membrane side, whereas the control side displayed little or no healing, even after 22 weeks of observation. GBR procedures have been commonly performed to increase alveolar bone for dental implant treatment since the early 1990s. The most commonly used GBR membranes in dentistry are made of resorbable collagen or non-resorbable expanded polytetrafluoroethylene (e-PTFE).

Inspired by GBR procedures, Tarchala replaced the IM with a synthetic PTFE membrane in a rabbit model of IMT. The results showed that the PTFE membrane displayed a similar osteogenic potential than the IM. Moreover, there was no difference in the amount of new bone in the defect site when the IM was replaced with the

synthetic membrane. PTFE is a non resorbable material. To streamline the IMT into a single stage surgery, other groups tested biologically derived and resorbable membranes: human amniotic membrane (hAM) and a commercially available human acellular dermis (Epiflex©). The first, hAM, is derived from human term placentas, a readily available clinical waste product. Furthermore, the clinical use of hAM in regenerative medicine, including ophthalmology and wound treatment, is considered ethically accepted. Gindraux et al. [7] were the first to describe biological similarities between IMs and hAM. The hAM is a very thin (no more than 0.5 mm thick) five-layer membrane rich in ECM proteins, including collagen, elastin, vitronectin, laminin and fibronectin. This membrane contains fibroblasts and amniotic MSCs, which secrete various growth factors (VEGF, EGF, FGF-2, PDGF and TGF-β), conferring on hAM its angiogenic and osteogenic properties. Proof-of-concept for the use of hAM in one-step IMT was obtained by Fenelon et al. [79]. They used radiographic scoring and µCT measurements to compare bone regeneration efficiency in rats after BMP-2/calcium-phosphate scaffold implantation between standard Masquelet PMMA-IMs and lyophilized or decellularized/lyophilized hAMs. Six weeks post-implantation, bone regeneration rates were similar in both the IM and decellularized/lyophilized hAM groups, albeit slightly lower in the lyophilized hAM group. The second membrane is Epiflex©, a clinically approved product for treating large burn wounds. Verboket and colleagues investigated the therapeutic effects of Epiflex on the healing of a large femoral bone defect in rats undergoing the transplantation of syngeneic spongiosa [80]. As expected, the use of the human acellular dermis leads to equivalent healing results in comparison to the two-stage IMT.

These experimental data highlight the potential of using GBR membranes as an alternative to Masquelet IM. However, few studies on this topic have been performed and additional experiments are required to check the efficacy of GBR membranes for the healing of critical-size bone defects. Another important issue that must be taken into account is the membrane material to be used. It is unknown whether a synthetic or a natural material would be most appropriate as a substitute for IM. In dentistry, the choice of GBR membrane materials (natural or synthetic, resorbable or non-resorbable) depends largely on the size and configuration of the bone defect. The performance of membrane materials in orthopedics probably also depends on the configuration and environment of the bone defect.

Effector-based strategies

Bone tissue-engineering materials for expanding or replacing the effector

Scaffolds are key components of bone tissue-engineering (BTE) strategies. Biomaterials such as polymers, ceramic or composite provide osteoconductive matrices during the tissue regeneration process. To improve the efficacy of the construct, some BTE approaches incorporate growth factors and/or cells to biomaterials [81]. The most widely used growth factors to create an osteoinductive environment are BMP-2 and BMP-7 (specific osteoinductive growth factors), PDGF (BMSC mobilization factor), VEGF (angiogenic growth factor), FGF-2 (factor indirectly stimulating osteoblast differentiation and angiogenesis), TGF-β1 (chemotactic agent involved in BMSC recruitment) and IGF-1 (promotor of BMSC differentiation into osteoblasts) [82–84]. BMSCs have been preferentially selected as osteogenic cells based on their ability to differentiate into either osteoblasts or chondrocytes. Adipose-derived mesenchymal cells (ADSCs) and periosteum-derived stem cells (PDSCs) can also successfully induce in vitro bone mineralization, albeit to a lesser extent.

In vitro evidence of bone mineralization is often considered compelling proof of the efficacy of bone-graft substitute approaches. However, the implantation of synthetic bone grafts into mammalian models of long-bone damage in vivo often results in poor efficacy for the promotion of bone healing. In an interesting multicenter analysis, Hulsart-Billström [85] examined the correlation between existing in vitro results and the in vivo outcomes observed for approximately 100 different bone biomaterials. They concluded that the correlation between in vitro and in vivo test results is poor for bone biomaterials. The presence of an inflammatory response in vivo, a lack of nutrient delivery to the injured bone and other physiological regulatory mechanisms affecting a given synthetic bone graft can undermine the efficacy of the in vivo mineralization as compared to the one observed in vitro for the same bone substitute cultured in a bioreactor under optimized conditions. But what about the efficacy of synthetic bone substitutes in the IMT context, in which the vascularized and cytokine-enriched IM creates an osteogenic environment around the graft?

BTE scaffolds investigated in animal models of IMT include calcium phosphate and carbonate derivatives, which have a chemical composition close to the natural mineral composition of bones. The efficiency of bone repair is lower following the implantation of a β-tricalcium phosphate (β-TCP) or 80% tricalcium phosphate (TPC)-20% hydroxyapatite (HA) composite alone into a PMMA- or

epoxy-induced IM in rats [86, 87] than after the insertion of a control synegeneic bone graft into the IM. However, the implantation of a β-TCP scaffold colonized with MSCs and EPCs [87] or a TPC-HA scaffold combined with a mixture of both BMP-7 and bisphosphonate (Zoledronate) results in bone healing at least as effective as that achieved with syngeneic bone grafts implanted into the IM [86]. Liu and coworkers used an alternative approach involving the production of a 3D-printed polylactic acid (PLA) and HA scaffold ("3D-printed PLA-HA") loaded with bone-marrow cells for the IMT-based repair of a bone defect in rabbit [88]. An evaluation of the healing process by μCT showed that new bone volume was greater in animals receiving an autologous iliac crest bone graft during the second stage of IMT (ICBG group) than in those receiving a 3D-printed PLA-HA scaffold (PLA-HA group). However, the addition of bone-marrow cells to the PLA-HA scaffold greatly enhanced bone repair to a level equivalent to that in the ICBG group.

In clinical practice, Gupta used a β-TCP ceramic as an autologous bone-graft expander [89]. Relative to ABG alone, the rate of non-union obtained with this material was higher only in patients who were smokers and in those with tibial bone lesions [89]. Piacentini et al. [37] experimented with the use of a mixture of autologous bone graft and frozen fresh cancellous bone allograft from a local bone bank combined with platelet-rich plasma (PRP) and concentrated bone-marrow aspirate subjected to calcium gluconate jellification. The results were disappointing, as the authors concluded that the healing rate (72%) observed with the enriched bone graft implanted in the second stage of IMT was lower than the rates typically reported for the standard ABG used in IM (bone union rate of 85–89%).

Other grafting materials investigated in human include Bioactive glass (BAG), which is considered of particular interest as it can enhance the osteoconductivity and mechanical strength of conventional hydroxyapatite (HA) and β-TCP ceramics. The osteoconductive properties of the BAG stem from the release of phosphate, calcium and silicium after implantation, causing the formation of a hydroxyapatite layer, leading to osseointegration and osteogenic activity. In addition, this release of ions induces a large local increase in pH and osmotic pressure, inhibiting bacterial proliferation and leading to antimicrobial activity. Van Vugt et al. [90] implanted a SiO_2-Na_2O-CaO-P_2O_5 BAG — S53P4 Bonalive© granules — in 69 patients with osteomyelitis. Nine of these 69 patients with severe infection were treated with a two-stage IMT protocol whereas the other 60 were treated with a one-stage procedure. Long-term bacterial eradication was observed in 85% of the treated patients. The authors found no relationship between the type of treatment (one-stage vs. two-stage treatment) and the recurrence of infection.

Furthermore, in a cohort of four patients, the use of a combination of S53P4 BAG with bone-marrow concentrate aspirate in a 79%/21% ratio was found to be effective against bone non-union in patients undergoing IMT [90]. In another study, eight patients with infected or non-infected large-bone defects underwent implantation with Bonalive© BAG granules with (5 patients) or without (3 patients) an autologous bone graft at stage 2 of the IMT. Bone healing was observed 16 months later, in all patients [91]. Building on the promising results obtained with this biomaterial, S53P4 BAG is currently undergoing testing at a larger scale in a clinical trial of 50 patients undergoing IMT in Germany. In the second stage, the bone defect will be filled with either bioglass (intervention group) or a mixture of ABG and a ceramic bone substitute (control group) [92].

Stimulation of the effector by the addition of growth factors

Attempts have been made to increase the osteoinductivity of ABGs by directly adding growth factors, such as BMP-7 or BMP-2. In animal models of IMT, the addition of BMP-7 to the graft material had a beneficial effect on bone healing in rats [86] and rabbits [93]. The results obtained for clinical applications of IMT are less clearcut. Haubruck et al. compared the clinical efficacy of autologous bone grafts combined with either recombinant human BMP-2 or BMP-7 for the treatment of lower limb bone non-union [94]. They showed that patients treated with rhBMP-2 had higher rates of bone healing than those who received rhBMP-7. By contrast, Masquelet et al. [95] reported disappointing result for the combination of rhBMP-7 with ABG in a series of 15 patients. The use of additional growth factors is associated with higher rates of delayed deformity and bone graft resorption. This failure may be due to possible effects of competition with growth factors secreted by the IM [23, 25].

Mechanical environment-based strategies

3D-printed cages for bone-graft retention

Innovative approaches based on custom 3D-printed metal cages have recently been developed to ensure rigid fixation at stage 2 and facilitate the osseointegration of the graft. Metals, including tantalum and titanium, have long been used for the manufacture of orthopedic implants for clinical use, given their excellent mechanical properties, particularly for load-bearing. Tetsworth was among the first to use 3D-printed titanium cages incorporating bone grafts for IMT [96]. In a small clinical cohort (5 patients), they used the mirrored CT images of the contralateral healthy limbs of the patients as templates for the printing of truss-type cage

implants. Titanium cages filled with morselized allogeneic and autograft cancellous bone were then inserted into the IM. Bone union was observed clinically and radiographically in all five patients. The cage constructs were mechanically robust and were strong and stable enough to allow immediate motion and early weight-bearing. The authors highlighted the importance of the two-stage IMT approach for the success of the procedure. The membrane induction period allows time not only for the healing of soft tissues and the eradication of infection, but also for completion of the design, manufacture, sterilization, and delivery of the custom implant. Consistent with the findings of this study, Gavaskar et al. [97] proposed a modified intramedullary nail called a "load-sharing nail-cage construct" for IMT stage 2. Basically, the ABG was compacted at the inner edge of a lumbar spine titanium cylindrical mesh cage. A central channel was left within the compacted graft to allow the passage of the interlocking nail. The resulting cage-nail graft construct was then inserted into the IM and the membrane was carefully sutured. This method gave a bone union rate of 100% in 21 patients with segmental tibial defects, as the load-sharing construct provides high stability, enhanced bone loading and protects the nail from axial and bending loads [97]. Many other groups have developed similar approaches for treating unstable fractures or fractures with complex geometries. For example, Foukas described an interesting custom-made titanium cage manufactured as a load-sharing bone scaffold and a bone-graft container for the healing of massive femoral bone loss in a young patient [98]. In another case study, Wu manufactured personalized 3D-printed porous tantalum prosthesis bonded to a locking plate to guarantee biomechanical stability for the reconstruction of infected femur defects. At stage 2 of the IMT procedure, the surgeons implanted the prosthesis without a bone graft into the IM cavity. Interestingly, 13 months later, radiological follow-up showed osdeointegration of the prosthesis and stable fixation, with deformity correction in all patients [99].

The use of 3D printing technology to improve the efficiency of the second step of IMT is promising in terms of improvements in therapeutic outcomes. However, longer-term investigations will be required to obtain clinical approval of such strategies, as these metal cages are designed to remain implanted for the entire life of the patient, promoting bone mineralization within their structure.

Ultrasound stimulation of the IM and the fracture site

Ultrasound is a form of mechanical energy as an acoustic pressure wave in biological tissues. The Food and Drug Administration (FDA) approved the use of low-intensity pulsed ultrasonography (LIPUS) as a noninvasive adjuvant treatment for promoting bone healing in 1994. The application of an ultrasound intensity of 30 mW/cm^2 to fresh tibial fractures for 20 min per day has been shown to significantly reduce healing time [100]. For delayed bone unions, LIPUS treatment improved healing rates when initiated within six months after the most recent operation [101]. LIPUS generates nanomotions at the fracture site that mediate signal transduction in the tissue.

The mechanism by which LIPUS treatment acts on bone healing has yet to be fully elucidated, but LIPUS is thought to promote bone healing through several different mechanisms. Ultrasound treatment improves the mechanical properties of bone during the healing process by increasing osteoid thickness and the bone mineral density of the newly synthesized bone [102]. LIPUS also modulates the inflammatory response early in bone healing process by increasing the synthesis of prostaglandin E_2 (PGE_2) and cyclo-oxygenase-2 (COX-2) [103]. In vitro, ultrasound treatment stimulates the proliferation and osteogenic differentiation of mesenchymal stem cells [104]. Other in vivo studies in the field of osteoarthritis have shown that LIPUS promotes collagen synthesis [105]. This effect is of particular interest given the collagenous structure of the IM. Based on these elements, a Japanese group used LIPUS to treat a patient suffering from a septic tibia fracture managed by IMT [106]. Four months after the second surgical union, bone union was progressing well and the patient was able to walk without pain. Further experimental studies performed in vitro by Takase et al. investigated the effects of LIPUS on BMSCs isolated from IM fragments [107]. LIPUS had no significant effect on cell proliferation, but this study found that ultrasound exposure promoted the osteogenic differentiation of the IM-derived MSCs and increased their alkaline phosphatase (ALP) activity. The authors argued that LIPUS could be used as an adjuvant tool for improving the healing process in IMT.

Conclusion

IMT has revolutionized the management of large-bone defects over the last three decades. It is more straightforward to perform than other surgical methods, and is effective due to the coordinated action of four components: an inducer (the PMMA spacer), a container (the IM bed), an effector (the graft) and an environmental modulator defined principally by the mechanical forces applied to the inducer-container-effector triad. In this review, we provide a comprehensive analysis of the established and hypothetical reasons for IMT failure and discuss various biotechnological strategies for optimizing surgical outcomes through modifications to these four components. One distinctive feature of this surgical technique is that it was discovered serendipitously and used for many

years by clinicians before attracting the attention of biologists. Diverse strategies for improving this technique have since been investigated. Future improvements to this approach will depend on the integration of innovations developed through collaboration between clinicians and researchers.

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Declarations

Competing interests The authors declare no competing interests.

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