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Review

BMP-2 Delivery through Liposomes in Bone Regeneration

Noemi Dirzu [1], Ondine Lucaciu [2,*], Dan Sebastian Dirzu [3,*], Olga Soritau [4], Diana Cenariu [1], Bogdan Crisan [5], Lucia Tefas [6] and Radu Septimiu Campian [2]

[1] Medfuture Research Center for Advanced Medicine, School of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania; noemidirzu@gmail.com (N.D.); diacenariu@yahoo.com (D.C.)
[2] Department of Oral Health, Iuliu Hatieganu University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania; rcampian@email.com
[3] Department of Anesthesiology and Intensive Care, Emergency County Hospital, 400006 Cluj-Napoca, Romania
[4] Ion Chiricuta Clinical Cancer Center, 400010 Cluj-Napoca, Romania; olgasoritau@yahoo.com
[5] Department of Maxillofacial Surgery and Implantology, Iuliu Hatieganu University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania; bbcrisan@yahoo.com
[6] Department of Pharmaceutical Technology and Biopharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, 400010 Cluj-Napoca, Romania; Tefas.lucia@umflcuj.ro
* Correspondence: ondineluc@yahoo.com (O.L.); dirzudan@gmail.com (D.S.D.)

Abstract: Bone regeneration is a central focus of maxillofacial research, especially when dealing with dental implants or critical sized wound sites. While bone has great regeneration potential, exogenous delivery of growth factors can greatly enhance the speed, duration, and quality of osseointegration, making a difference in a patient's quality of life. Bone morphogenic protein 2 (BMP-2) is a highly potent growth factor that acts as a recruiting molecule for mesenchymal stromal cells, induces a rapid differentiation of them into osteoblasts, while also maintaining their viability. Currently, the literature data shows that the liposomal direct delivery or transfection of plasmids containing BMP-2 at the bone wound site often results in the overexpression of osteogenic markers and result in enhanced mineralization with formation of new bone matrix. We reviewed the literature on the scientific data regarding BMP-2 delivery with the help of liposomes. This may provide the ground for a future new bone regeneration strategy with real chances of reaching clinical practice.

Keywords: BMP-2; liposomes; drug delivery; growth factors; osseointegration; implantology; transfection

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1. Introduction

In maxillofacial and orthopedic research, bone regeneration represents one of the main focuses. Critical size bone tissue loss resulted after trauma, infection, tumors, systemic diseases, osteoporosis, or surgical resection and often need consolidation or replacement using different biomaterials, autografts, allografts, or xenografts [1–3]. Non-union fractures can account for up to 12 percent of all fractures and carry the risk of complications such as severe pain or loss of function, as well as prolonged hospitalization which results in higher costs [4].

Archeological findings attest to the replacement of missing teeth starting with the ancient Egyptians and the Mayan civilization [5]. Since 1949, when Goldberg and Gershkoff published the first scientific article describing the use of metals as dental implants [6], novel biomaterials and surgical approaches have made dental implantation an everyday procedure and, today, millions are performed every year with success [5]. Underlying periodontal disease [7], insufficient bone and a long, difficult recuperation period before the patient can fully regain use of function, are the main challenges in cur-

rent implantology. During this time, the patient must comply with a series of recommendations which drastically impact the quality of their life. If periodontal disease is present, the underlying bone often has a poor quality, and implants fail to integrate. A good osteoinductive material has a role in support, as well as the capability to recruit mesenchymal stromal cells (MSCs), deliver the growth factors which are necessary in the differentiation process, and rendering a faster, enhanced bone formation [8,9].

In bone remodeling, the main growth factors are the members of the TGFβ superfamily, mainly bone morphogenetic proteins (BMPs) [10–13]. The delivery of these factors is a problem not yet solved. In the literature, numerous drug delivery systems are described but concerns are that none of these are ideal. Adding growth factors to metals increases the price of the implants significantly. Delivery by calcium-phosphate ceramics increases the necessity of growth factors and there is a risk for factor degradation [14]. Liposomes are bioactive vesicles which can encapsulate many types of molecules and specific genetic sequences that transfected into the cells can increase the secretion of specific proteins. They are excellent carriers, highly biocompatible, but overlooked in many areas, such as bone regeneration. Including growth factors in liposomes, reduces the amount used, and subsequently the costs and the risks of side effects [12].

In this review article, the aim is to compile the evidence regarding the use of liposomes as growth factor delivery systems in bone regeneration, most specifically, in the osseointegration process of dental implantation.

2. Components of Bone Regeneration

For either the insertion of a simple screw or the most complicated implant, it is imperative to find better and faster bone regeneration techniques [15]. Stem cells, biomaterials, and bioactive molecules are the major factors in current bone regeneration research.

2.1. Cells

Osteoblasts and osteoclasts are the two major cellular elements in bone remodeling. Osteoclasts attach to the old bone and reabsorb the damaged tissue, while osteoblasts migrate to the lesion site attracted by the secreted cytokines and fill up the gap created by the bone resorption [16]. In regenerative research, MSCs are used to obtain osteoblast, as they can differentiate into diverse cell types depending on the external stimuli. They have been defined as a population of plastic adherent, stem-like cells which can be isolated from various tissues [17]. These cells have the potential to differentiate into osteoblasts [18], chondroblasts [19], pancreatic cells [20], adipocytes [21] or myocytes [22] depending on the molecules of the micro medium in which they are seeded. They were first isolated from bone marrow, but lately were retrieved from numerous tissues, such as umbilical cord [23], adipose tissue, as well as healthy and diseased oral structures [8,24,25]. BMPs induce differentiation of MSCs into pre-osteoblasts and mature osteoblasts [26].

Osteoblastic differentiation requires the expression of two main transcription factors: Runx2 and osterix (Osx) [27]. Stem cells have the ability to migrate to the site of bone regeneration and secrete different biomolecules. Expression of Runx2 is low in MSCs, but rises when the cells differentiate and start to secrete BMP-2 [28–30]. Runx2 also encodes for other cellular markers involved in osteochondral calcification, such as alkaline phosphatase (ALP) [31], osteocalcin (OC), and osteopontin (OP) [32]. Transgenic mice which lack Runx2 have completely cartilaginous skeletons [33], because they do not have osteoblasts and mineralization does not occur [34,35]. Similarly, mice which lack Osx, have perfectly structured skeletons, but without ossification. The Osx gene acts downstream from Runx2. Mice lacking Runx2 do not express Osx, but those missing Osx can have Runx2 intact, therefore, many consider Runx2 as the main gene in osteoblastic differentiation [36,37].

Osteoinduction is the cellular process during which an undifferentiated osteoprogenitor cell transforms in bone tissue under the influence of the local environment.

2.2. Biomaterials

There are many materials used in dental medicine, from the simplest suture thread to the most complicated implants. Biocompatibility is a paramount aspect of any material which comes in contact with tissues. Dental implants are made from various materials such as titanium, bioceramics, composites, natural and synthetic polymers, carbons [38–42], and their combination. Every one of these materials has advantages, but also disadvantages, and the perfect implant is yet to be invented [43]. From all the biomaterials, the closest to ideal is titanium, a versatile metal used both in pure form and as an alloy. Its superior biocompatibility is due to the fact that it is a highly reactive metal which has a high affinity to oxygen and spontaneously forms a very stable oxide layer at its surface in less than a millisecond after exposure to atmosphere [44]. Studies have shown a seven year survival rate of titanium implants between 94.6–95.7% [45–47]. The main disadvantage of titanium is represented by its high modulus of elasticity, which is five to ten times greater than the underlying human cortical bone in which it is implanted [48]. Because of the difference between the two, bone is resorbed, the implant loosens, and revision surgery is needed [49].

With the purpose of enhancing the success rate and reducing the osseointegration time, research has focused on improving the interface between the organism and the inorganic substrate by functionalization of the implant with different biolayers and biomolecules. The surface of the implant is the part which interacts with the recipient tissue and for better results we need to fully comprehend all the mechanical, physical, and chemical interactions that take place here [50,51].

2.3. Growth Factors

The most important class of biomolecules in bone regeneration are the bone morphogenetic proteins (BMPs), members of the TGFβ superfamily [26,52]. They play a fundamental part in differentiation, embryonic development [53], and even tumorigenesis and cancer progression [54,55]. BMPs were first identified by Urist, in 1965 [56,57], and purified by Wang et al. two decades later [58]. After the identification and cloning of the BMP genes the manufacturing of recombinant human BMPs followed [59]. There are 20 different types of BMPs described in the literature as markers of the osteogenic cell differentiation process. BMP-2 and BMP-7 are currently approved by the FDA for human use [52,60]. BMP-2 is the most potent molecule in osteoinduction, essential for new bone formation. During fracture healing, the molecule is released during the degradation of the bone by osteoclasts and acts like a beacon for MSCs to find the lesion sites. During in vitro experiments, it is added to the culture media in the process of differentiation of stem cells into osteoblastic lineages for a better and faster osteogenic transformation. Attempts to replicate this in vivo frequently fail, because growth factors have a short half-life, and they are rapidly removed from the lesion site by the circulatory system. Human recombinant BMP-2 (rhBMP-2) is expensive, and its stability and biological activity in vivo is limited [61]. Off label use results, sometimes, in significant complications such as dysphagia [62], airway swelling [63,64], ectopic and heterotopic bone formation [65,66], immune response, or tumorigenesis [67]. For this reason, we have to optimize its intake by the cells by adding them to slow-release delivery systems. Pre-differentiation of MSCs, produces BMP-2 and attracts the organisms own stem cells to the injury site [68,69]. There are no standards of dosage for in vivo animal studies, the use of BMP-2 for cartilage and bone regeneration ranges between 0.015 and 150 μg/implant and even the FDA-controlled formulations are used in supra-physiological doses [70].

2.4. Growth Factor Delivery Systems

The major problem related to the in vivo use of growth factors in bone regeneration is represented by the lack of a reliable administration method [71]. In order to modulate osteogenic differentiation, different strategies can be applied. Systemic delivery methods often fail because of the accumulation of the growth factors in the kidneys and subsequent elimination. Injection to the site of the injury is most often used, but the lack of a good vessel leads to absorption into the systemic circulation and elimination. Specificity for a certain organ or even cell type is very important, and the lack of it is the biggest drawback of systemic drug delivery. Different delivery systems have been used to obtain a slow, controlled release of active molecules. Protection of the active molecule can be done by inclusion in carriers [72] or fixating them to the surface of the implant [73]. Biocompatibility is one of the most important features of the carriers. Natural compounds, i.e., collagen [74], gelatin [75], fibrin and fibronectin [76], chitosan [77], hyaluronic acid [78]; synthetic polymers, i.e., poly(lactic acid) [79], poly(glycolic acid) [80], poly(lactic-co-glycolic acid) [81]; or inorganic materials, i.e., hydroxyapatite (HA) [82,83], tricalcium phosphate (TCP) [84], and combinations of them are used for slow delivery.

3. Liposomes

Liposomes are biocompatible, self-assembled, spheric vesicles composed of concentric phospholipid bilayers wrapped around an aqueous compartment. Phospholipids are composed of a hydrophilic polar head and a hydrophobic non-polar tail. They were first described in the 1960s [85] and have been used as delivery systems since the 1970s [86]. As their structure is similar to cell membranes, they can easily merge with cells, penetrating them, and discarding their cargo. They can incorporate many types of molecules depending on the structure of the liposome and the hydrophilicity of the entrapped molecule. The hydrophilic drugs are entrapped into their inner compartment and the hydrophobic ones are linked to them directly or indirectly, either on the surface or between the two lipid layers (Figure 1). They are extremely versatile. Although there is little researched on their role in bone regeneration, their role in other areas is well documented. Liposomes are used as a vector in more than 20% of approved clinical trials in controlled drug delivery [87]. There are numerous FDA-approved clinical applications of liposomes in vaccine development, as well as antibiotic and analgetic delivery [88]. In cancer therapy, antibody conjugated liposomes (immunoliposomes) have been used in clinical trials to eliminate circulating cancer cells, preventing metastasis [89]. In the COVID-19 pandemic, the first approved vaccines were using liposomal transfection of messenger RNA [90]. By conjugating with magnetic nanoparticles and imaging agents, in vivo traceability of liposomes has been achieved. Targeted release has been obtained by exposure to ultrasound [91] or magnetic actuation [92,93]. Using a combination of radioactive molecules bonded to liposomes, diagnostics, targeting, and treatment have been obtained, which has been called theranostics [94].

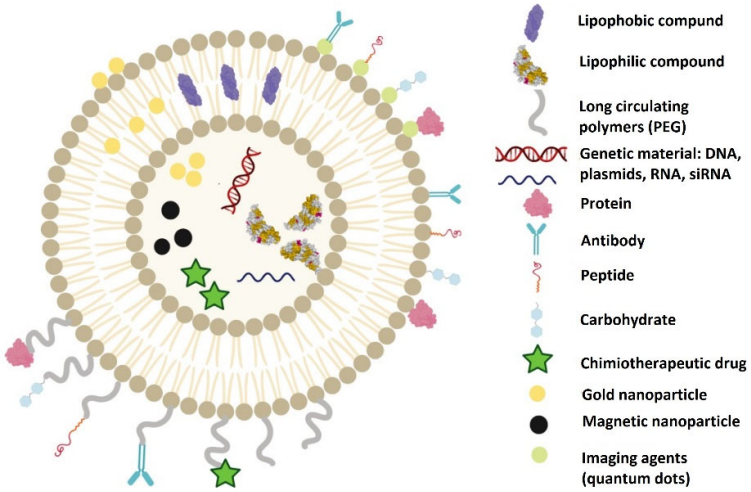

Figure 1. Structure of liposomes and the molecules that can be delivered through them.

Attempts were made to immobilize liposomal BMP-2 onto different biocompatible scaffolds for a sustained, long-term release. Electrospun poly(L-lactic acid) fibers, functionalized with hydroxyapatite (HA) can be a good carrier for BMP-2 loaded liposomes. Adipose tissue-derived MSCs were seeded onto the scaffolds. The levels of ALP and calcium ions were significantly higher in the liposome and HA containing scaffolds than in the control group (HA-coated scaffolds with free BMP-2). The expression level of the genes related to osteogenesis were three-fold as compared with the control group. The osteoconductivity of the constructs was tested in vivo by subcutaneous implantation into rats. At the site of the implants, MSCs aggregated and primary ossification centers appeared [95].

Magnetic liposomes have also been used to carry BMP-2 and in combination with magnets and have had good results in maintaining the proteins at the injury site for a prolonged period of time. Entrapment efficiency was approximated to be the same with fluorescein isothiocyanate-conjugated dextran (FD-40) which has the same molecular weight as BMP-2. The magnetic liposomes had entrapped a lower quantity of FD-40 than the conventional ones. A critical size bone defect was created in the animal's femur and a magnet was inserted. Different magnetic and non-magnetic liposomes were injected at the injury site, at different timepoints. Only the animals injected with magnetic liposomes immediately after the surgery, presented complete bone bridge formation [96].

In situ gels are a good alternative for drug delivery systems. They are liquid ex vivo and turn into gels in the organism depending on several factors. Growth factors included in liposomes can be entrapped in gels for a prolonged and controlled release, resulting in longer and more stable plasma levels of the protein and significantly more bone formation when injected into critical size bone defects [97].

Hydroxyapatite is found only in bone tissue and designing systems that can link to it is an important goal in osteogenetic research. Bisphosphonates (BPs) are ligands with a high affinity to osseous tissue, which prevent bone resorption by impairing the function of osteoclasts [98,99]. They can be conjugated with active molecules such as BMPs. Produced by two methods, Wang et al. found that the BP micelles and BP liposomes had a strong affinity to HA vs. the PEGilated ones, while the in vitro and in vivo bone-inducing capacity of BMP-2 was maintained [100]. The studies from the literature are detailed in Table 1.

Table 1. Direct liposomal administration of BMP-2.

Molecule	Liposome/Scaffolds	Cells	Entrapment Efficiency	Animal intervention	Examination	Citation
BMP-2	HSPC DSPC DPPC Chol mPEG$_{2000}$-DSPE Film hydration Electrospinned PLLA Nanofibers with HA covering	Human adipose MSCS	Maleimide quantification assay Confocal laser scanning microscopy FE-SEM (field emission SEM) Osteogenic differentiation Cytocompatibility and proliferation ALP activity Gene expression analysis RunX2, OC, GAPDH RT-PCR	Male Wistar rats Subcutaneous pocket for ectopic bone formation	Histology, hematoxylin-eosin	[95]
rhBMP-2	Magnetic liposomes	-	Fluorescence spectrophotometry Dynamic light scattering TEM	31 Male Sprague Dawley rats with critical size bone defect in the femur	Radiography once a week for 9 weeks Microcomputed tomography Histology Mechanical testing by torsion	[96]
rhBMP-2	Multilamellar Included in gel	-	Spectrophotometer at 280 nm	30 New Zealand rabbits Maxillary critical sized alveolar defect	Plasma levels of BMP-2 by ELISA Histology, HE light microscopy Quantitative histomorphometric analysis	[97]
rhBMP-2	PEG BP	Human C2C12 Rat BMSC	Doxorubicin model for encapsulation Lipophilic fluorescent tracer MTT cytotoxicity Bioactivity assay, ALP Spectroscopy In vitro HA binding assay	Female Sprague Dawley rats		[100]

4. Transfection

Gene therapy is used to transfer genetic material into specific cells to obtain the secretion of a certain protein. DNA, plasmids, siRNAs, miRNAs can be delivered into target cells using vectors which protect them and facilitate their transport through the cell wall [101,102]. This method may be more effective than the exogenous utilization of the molecule, because it restricts the migration of the molecule and the accumulation in other organs. Transfection of DNA into cells is the ideal way to study some functions of proteins. The cells will become, by this method, protein producing factories at the site of the injury. Viruses represent a good vector, as they developed natural ways to enter the host cell and integrate in their genetic material. Adenoviruses, retroviruses, adeno-associated viruses, and lentiviruses are used as vectors the most efficiently [103–105], but they carry significant side effects and limitations, such as inflammatory and immune reactions, limit of included DNA size, or certain tumorigenic mutations [15,106–108].

Non-viral vectors are mostly cationic polymers or cationic liposomes, which interact with the negatively charged genetic material and can be transported into the cell. Liposomal transfection is not as efficient as the viral ones [109], but it yields a series of advantages such as lack of immune response, toxic byproducts, ectopic bone formation, and accumulation in organs [67]. Liposomes are a viable solution for transfection of large genetic structures, as their capacity to carry genetic material is not limited by size [110].

The genetic sequence is first loaded into the liposome. After the construct enters the cell, the gene is released, it enters the nucleus, where it integrates into the cell's DNA. Thus, the cell produces BMP-2 (Figure 2).

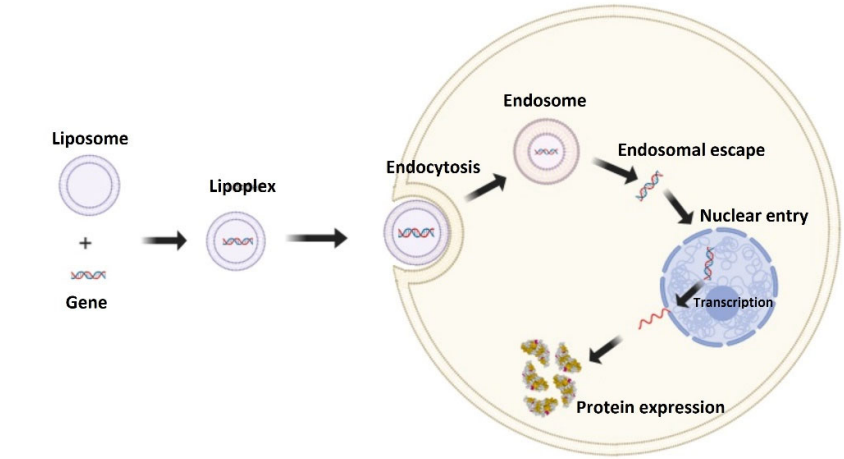

Figure 2. Mechanism of liposomal transfection.

There are very few studies in the literature in which direct loading of BMP-2 is used; however, more studies concentrate on transfection of BMP-2 genes into cells.

Park et al. conducted a study on pigs where they created calvarial bone defects in which they inserted implants with or without BMP-2 transfected liposomes. Previously, they had assessed the transfection efficiency of the liposomal vector by introducing green fluorescent protein. The osteogenic capacity was measured at 7 and 28 days and at three different regions of interest. The bone regeneration was significantly enhanced in

the group with the liposomal vector applied to the surface of the implant. The direct application of the vector was sufficient for complete bone healing at the margins of the bone defect, but not in the center [111]. Using collagen for the liposome/BMP-2 carrier allows migrating cells to express the protein even after 28 days [13].

Neo-angiogenesis is one of the main components of bone regeneration and, at the same time, one of the biggest challenges. For the formation of new bone, it is important for nutrients to be provided, the acid-base balance to be maintained, and the metabolic by-products to be eliminated. The combined release of BMP-2 and VEGF could solve this issue [112–114]. Xiao-bin et al. evaluated the efficacy of transfection of mouse bone marrow stromal cells (mBMSC) with BMP-2 and $VEGF_{165}$ in order to assess the neoangiogenic and ectopic bone tissue forming capability of these molecules. They found co-expression of BMP-2 and $VEGF_{165}$ mRNA in vitro by immunohistochemistry and RT-PCR. In mice, they obtained ectopic trabecular-like bone formation at 4 weeks after injection [115]. Guo-ping et al. researched, in vitro, the transfection efficiency of a vector which co-expressed hBMP-2 and $hVEGF_{165}$ and the resulted protein levels. They found that the transcription of hVEGF may be upregulated by hBMP-2 by RT-PCR analysis of the proteins. Western blot did not show this cooperativity. Osteocalcin mRNA and collagen I were high in the groups transfected with BMP-2, but negligible in the groups with VEGF alone [116].

When comparing liposomes with polyethylene glycol (PEG) as gene carriers, BMP-2 levels in cells and mRNA levels of BMP-2 were double in PEG group than in liposomal group. The liposomal group was only used as control in vitro, but not in vivo [117].

Liposome-loaded DNA have been introduced in multilayer HA coatings deposited on titanium disks using the layer-by-layer technique. The amount of DNA was increased by each additional layer, the plasmids were released, and cells were transfected, with an increased expression of Runx2, Osx, ALP, and OC, but without calcified nodule formation at 14 days [118]. At implantation in rabbits, the uncoated implants yielded new woven bone, showing a statistically significant difference at 4 weeks (but not at 2 or 8 weeks) in favor of the BMP-2 gene coated implants. However, the bone-to-implant contact was consistently lower than in the control group, which the authors explained by the short persistence of the protein at the site [119].

PEG membranes are biodegradable materials often used in bone tissue engineering. In adult pig experimental model, PEG membranes containing liposomal BMP-2 transfected osteoblasts facilitated a significantly higher new bone regeneration, cell survival, and protein synthesis at 1, 2, 4, and 8 weeks after implantation. The combination of PEG matrixes and osteoblasts transfected with BMP-2 allowed for a good spatial fixation of the implanted cells in the defect [120,121].

Kroczek et al. compared the effect of BMPs to other members of the TGFβ superfamily such as TGFβ and IGF1. They transfected the genes into BMSCs, implanted the cells into mini-pigs, and evaluated the bone formation. Cells transfected with TGFβ and IGF1 did not enhance bone formation as compared with the negative control, while those with BMP-2/7 yielded good quality bone tissue with enhanced mineralization and organized architecture [122].

The extracellular matrix of bone consists of 70–90% of hydroxyapatite (HA) and 10–30% organic material, mainly collagen [123]. HA ceramics have been used as a substitute for autologous bone or as a carrier for bioactive molecules. Adding liposomal BMP-2 cDNA to HA scaffolds leads to better bone formation than HA alone or liposomal BMP-2 alone. The BMP-2 expression was present at 3 and 6 weeks after which it decreased gradually [124]. Human amnion mesenchymal stem cells (hAMSC) transfected with BMP-2 in a liposomal formulation seeded on nano HA/collagen/poly(1-lactide) had a similar proliferation and differentiation capability as those cultured in osteogenic culture media. The cells transfected had higher expression of OC and Runx2 [125].

Recently, stem cells of buco-maxillar origin have been identified and isolated [8]. Dental follicle, alveolar bones, and ligaments have proven to be excellent sources of stem

cells. They are readily available from discarded medical waste and have proven to be superior in osteogenesis as compared with stem cells of other origins. The periodontal ligament plays an important role in stability, nutrition, and regeneration of the teeth. Stem cells isolated from it have been successfully used for differentiation into osteoblasts when transfected with BMP-2 plasmids using a liposomal vector [126].

The studies conducted on liposomal delivery of BMP-2 through transfection are detailed in Table 2.

Table 2. Transfection of BMP-2 genes.

Molecule(s)	Liposome/ Substrate	Cells Used	In Vitro Testing	Animal Intervention	Examination	Citation
hBMP-2 (pCMVBMP-2 plasmid)	NA	Pig BMSC	Immunohistochemical staining for BMP-2 Green fluorescent protein (GFP)	8 Pigs with calvarial peri-implant bone defects	Biopsy at 7 and 28 days Microradiography Immunohistochemistry	[111]
BMP-2	NA	-	-	8 Domestic pigs with calvarial defects	Microradiography Masson–Goldner trichrome staining, light microscopy	[13]
BMP-2/$VEGF_{165}$	pIRES	Mouse BMSC	RT-PCR: BMP-2 and VEGF165 Immunohistochemistry	4 Male nude mice, injection in thigh muscle poach	Digital radiography for ectopic bone formation at 4 weeks hematoxylin-eosin staining	[115]
hBMP-2 hVEGF165	pIRES	Human BMSC 14 days	RT-PCR: BMP-2 and VEGF165 Western blot: BMP-2 and VEGF165 RT-PCR: of OC mRNA Immunohistochemistry: Collagen I ALP activity assay	-	-	[116,127]
hBMP-2	NA	Rat BMSC	GFP In situ hybridization Immunohistochemical staining: OC, collagen I, OP RT-PCR Alizarin red: Calcium deposits	Rat mandibular critical size defect	Trichrome–Goldner staining Immunohistochemical staining, OC	[67]
	NA	Rabbit BMSC	Fluorescent microscopy Flow cytometry: Cell cycle analysis Western blot Q-PCR: mRNA BMP-2, OC Immunohistochemistry ALP activity assay	-	-	[128]
BMP-2 + BMP-2/EGFP	NA	Mouse pre-	Hoechst 33258: DNA	15 New Zealand white	Fluorescent labeling:	[119]

		osteoblastic MC3T3-E1 cells	Immunofluorescence Alamar blue: Cell viability	rabbit femur	Oxytetracycline hydrochloride 7 days Alizarin-complexion at 28 days Calcein green at 46 and 53 days Histomorphometric analysis	
rhBMP-2	NA	Mouse pre-osteoblastic MC3T3-E1 cells	Fluorescence: Nucleic acid labeling- ELISA: BMP-2 Alamar Blue: Cell morphology, attachment and proliferation Hoechst 33258: Phalloidin (actin), DNA ALP activity assay from cells OC from culture media Alizarin red: Calcium deposits Q-PCR: Runx2, ALP, OC, Osx	-	-	[118]
BMP-2 BMP-4	HA/TCP	Human fetal osteoblasts	-	15 Domestic pigs with frontal skull monocortical critical size defect	Histological sections, toluidine blue O Immunohistochemical staining for BMP-2/4, ALP, and V5	[120]
BMP-2	PEG		-	20 Domestic pigs with critical size defect in frontal skull	Histological sections, toluidine blue O Immunohistochemical staining for BMP-2/4 and Sox9 V5-tag,	[121]
BMP-2 BMP-7 TGF-β IGF-1		BMSCs	-	24 Skeletally immature Goettingen mini pigs	BMP-2 and OC expression, immunostaining Histological analysis Micro radiological analysis	[122]
BMP-2 + GFP			-	3 Adult pigs withfrontal bone defect	Expression of GFP and BMP-2 Immunohistochemistry: Semiquantitative evaluation of GFP and BMP2	[129]
BMP-2	HA		-	36 Japanese white rabbits with craniotomy	Histopathology: Cole, hematoxylin-eosin Immunohistochemistry: BMP-2	[124]
rhBMP-2	HA/Col l/PLA	hAMSCs amnion	SEM Osteoblastic differentiation: ALP,	-	-	[125]

		alizarin red, calcium phosphate, OC Q-PCR: OC, Runx2 Western blot MTT: Cell proliferation			
rhBMP-2/EnhancedGFP	Periodontal ligament cells	Western blot ALP activity assay Q-PCR: BMP-2, Runx2, Col type I, BMP-2, OC MTT assay: Cell proliferation Alizarin red staining: Calcium deposits	-	-	[126]

Comparative studies of efficiency of different vectors of BMP transfection.

Blum et al. compared the activity of luciferase one day after performing adenoviral, retroviral, and lipiosome mediated BMP-2 transfection into rat MSCs. The reporter gene was delivered efficiently by all three vectors. They obtained the best results using adenoviruses [104].

Park et al. compared liposome- and adenovirus- mediated gene transfer of BMP-2 cDNA in rat BMSCs and transplanted these cells into periosteal tissue. Gene expression lasted more than 14 days using either method, but adenoviral transfer resulted in double the amount of positive cells. In vivo healing of critical size bone defects by liposome-mediated gene transfer was slower, but the new bone had a normal configuration and physiologic orientation as compared with the adenoviral group in which the bone was significantly thicker [67]. In cartilaginous regeneration, liposomal transfection is also less efficient, forming only low rigidity fibro-cartilaginous tissue. Cells transfected by adenovirus formed tissue similar to hyaline cartilage [130].

5. Conclusions

In this review article, we detailed the existing BMP-2 delivery systems by liposomes. In the literature we found two methods described: direct addition of the growth factor and transfection through gene carrying. There are hardly any studies in which BMP-2 was added directly into liposomes, but the existing studies report good results both in vitro and in vivo, on animal studies. More papers are written on transfection of BMP-2 gene carrying liposomes. In vitro experiments show an excellent transfection efficiency by liposomes [111]. Combination therapy with VEGF yields an improved osteogenic differentiation [115,116], while the combination with TGFβ and IGF1 do not enhanced bone formation [122]. Animal studies are also promising, showing enhanced mineralization [119,122] and spatial fixation [120,121]. Due to these encouraging results, we anticipate that delivery of BMP-2 by liposomes will gain terrain in bone regeneration research. There is much need of better bone regeneration techniques, but we are still far away from the point where clinical translation is to be achieved before application of the method to humans. Future research has to establish the right dosage of BMP-2 delivery. In addition, in this review, we did not find studies comparing the two methods of BMP-2 delivery by liposomes.

Liposomes seem to be a good carrier for BMP-2. They enhance the osseointegration quality and shorten the required time. However, further investigation is needed in this area to properly translate it to clinical settings.

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Data Availability Statement: No informed consent was required as it did not present any identifiable data.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

TGFβ	Transforming Growth Factor β
MSC	Mesenchymal Stromal Cell
BMP-2	Bone morphogenetic protein 2
rhBMP-2	recombinant human BMP-2
Runx2	Runt-related transcription factor 2
Osx	Osterix transcription factor
ALP	Alkaline Phosphatase
OC	osteocalcin
OP	osteopontin
FDA	Food and Drug Administration
HA	hydroxyapatite
TCP	tricalcium phosphate
DNA	deoxyribonucleic acid
siRNA	small interfering ribonucleic acid
miRNA	micro ribonucleic acid
VEGF	Vascular Endothelial Growth Factor
hVEGF	human Vascular Endothelial Growth Factor
hBMP-2	human Bone morphogenetic protein 2
BMSC	Bone Marrow Stromal Cell
mRNA	messenger ribonucleic acid
RT-PCR	Revers Transcription Polymerase Chain Reaction
Peg	polyethylene glycol
IGF1	Insulin growth factor 1
cDNA	complementary deoxyribonucleic acid
Q-PCR	quantitative PCR
BP	bisphosphonates
GADPH	glyceraldehyde 3-phosphate dehydrogenase

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