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Microfluidic PLGA Microcapsules for the Sustained Delivery of Recombinant Human Bone Morphogenetic Protein 2 in 3D Printed PCL/βTCP Scaffolds

Sara M. Kasten
University of Pennsylvania School of Dental Medicine, saramkasten@gmail.com

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Abstract
Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a clinically available osteoinductive growth factor. In its current form, approved for clinical use, however, the growth factor is delivered in excessively high doses, resulting in unpredictable bone growth and unwanted, sometimes life-threatening, clinical side effects. It has clearly been demonstrated that the sustained, long-term release of the proteins can lead to improved ossification, owing particularly to the rapid metabolism of the biologically active growth factor when delivered alone in solution. Delivery systems for rhBMP-2 have been investigated extensively; yet still, further exploration into the best means of protein delivery — in order to curb the side effect profile and improve the quality of the bone generated — is warranted. The present review of literature introduces extensive work evaluating optimal rhBMP-2 delivery, with particular focus on natural polymers, inorganic materials, as well as synthetic materials. In particular, poly(DL-lactide-co-glycolide) (PLGA) microspheres, conventionally fabricated by a solvent extraction/evaporation procedure, are discussed for their use in sustained drug delivery. The application of rhBMP-2 for bone tissue engineering holds great promise for researchers and clinicians in both the medical and dental fields; yet, there remains a need for further investigation into improved protein delivery mechanisms.

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Hyun-Duck Nah, D.M.D., Ph.D.

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LITERATURE REVIEW

Sara Malenbaum Kasten, D.M.D.

Hyun-Duck Nah, D.M.D., Ph.D.

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University of Pennsylvania School of Dental Medicine

Children's Hospital of Philadelphia

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ABSTRACT

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a clinically available osteogenic growth factor. In its current form, approved for clinical use, however, the growth factor is delivered in excessively high doses, resulting in unpredictable bone growth and unwanted, sometimes life-threatening, clinical side effects. It has clearly been demonstrated that the sustained, long-term release of the proteins can lead to improved ossification, owing particularly to the rapid metabolism of the biologically active growth factor when delivered alone in solution. Delivery systems for rhBMP-2 have been investigated extensively; yet still, further exploration into the best means of protein delivery — in order to curb the side effect profile and improve the quality of the bone generated — is warranted. The present review of literature introduces extensive work evaluating optimal rhBMP-2 delivery, with particular focus on natural polymers, inorganic materials, as well as synthetic materials for protein delivery. In particular, poly(DL-lactide-co-glycolide) (PLGA) microspheres, conventionally fabricated by a solvent extraction/evaporation procedure, are discussed for their use in sustained drug delivery. The application of rhBMP-2 for bone tissue engineering holds great promise for researchers and clinicians in both the medical and dental fields; yet, there remains a great need for further investigation of improved protein delivery mechanisms.

REVIEW OF LITERATURE

The treatment of skeletal defects within the craniofacial region poses a significant challenge for plastic surgeons, oral and maxillofacial surgeons, and dental professionals alike. Such defects can be the result of trauma, disease, or congenital deformities — such as cleft lip and palate — and can lead to disfigurement, and loss of ability for normal function.[1] Depending on defect size, tissue repair is possible; but in defects beyond a critical size, bone grafts are often necessary. Autogenous bone grafts remain the optimal, "gold standard" of treatment; however, there is significant post-op pain and donor site morbidity,[2, 3] as well as increased costs and limited donor site bone availability, leading investigators to seek out alternate graft sources.[2]

Bone Morphogenetic Proteins

Recombinant human bone morphogenetic proteins (rhBMPs), key cytokines in bone formation and repair, have been evaluated for their use in the treatment of craniofacial defects.[2, 4, 5] Bone tissue engineering involves the delivery of an osteoinductive growth factor—such as BMP—at the site of the defect. BMP-2, BMP-4, BMP-7, and BMP-9 are members of the transforming growth factor beta (TGF-β) superfamily, and have demonstrated the capacity to induce progenitor cells from mesenchymal sources to differentiate into osteoblasts.[4] They play a role in osteogenesis, chondrogenesis, angiogenesis, as well as mesenchymal stem cell chemotaxis.[4, 6]

BMPs play a critical role in cellular functioning and the formation of new tissues; however, the most effective means of delivering the growth factor to the site of repair has yet to

be determined. When administered as a bolus dose, rapid dispersion throughout the body can result in undesirable side effects such as local inflammation, soft tissue edema, ectopic or heterotopic bone formation, and seromas.[7-9] BMPs have a very short half-life; therefore, when administered in solution, much of the bioactive protein is rapidly lost, leaving insufficient amounts of protein to act at the intended site.[10]

Current clinical applications:

Currently, the growth factor is approved for clinical use in a few select applications. In the form of the INFUSE bone graft, rhBMP-2 has been approved by the U.S. Food and Drug Administration as a medical device for use in spinal fusions, sinus lift procedures, and repair of alveolar defects after dental extractions.[11] Approval for the clinical delivery of rhBMP-2 with INFUSE was first granted by the FDA in 2002, for delivery of the growth factor in an adsorbable bovine type I collagen sponge (ACS).[11] There are, however, drawbacks of this application; the most striking of which is the extreme, supraphysiologic dose of protein required for the desired osteogenic effect.[12, 13] It is noted that the amount of rhBMP-2 present in one INFUSE dose for a single spinal fusion exceeds the amount of exogenous BMP naturally present in 1000 humans.[13] Nonetheless, rhBMP-2 is still combined with adsorbable collagen sponges for clinical use.

A retrospective cohort study from the Journal of the American Medical Association evaluated 328,468 patients undergoing spinal fusion procedures between 2002 and 2006.[14] They reported an extreme increase in the use of the growth factor from 0.69% to 24.89% during the four year period. In addition, higher rates of complications such as dysphagia, edema, hematoma, and respiratory problems, were reported when BMP was used in anterior cervical

fusions. The use of BMP was also correlated with increased length of hospital stay and higher hospital charges.[14] The authors were the first to compile national data on the use of BMPs in spinal fusion surgery, and successfully highlighted the widespread use of the growth factor clinically and the concerns of many medical professionals.

In addition, the growth factor is often used off-label in other anatomical sites such as alveolar clefts or cranial vault reconstruction.[15] Alonso et al.[16] compared the delivery of rhBMP-2 in an adsorbable collagen sponge matrix to a traditional iliac crest autogenous bone graft (ABG) in cleft palate patients aged 8-12 years, all with pre-operative orthodontic maxillary expansion. Results illustrated alveolar bony bridging, as well as normal dental eruption, in all patients. Overall average bone volume was slightly less in the treatment group as compared to the ABG patients, but clinical results were favorable in both groups. The authors noted that the decreased structural stability of the collagen sponge carrier was problematic. Additionally, post-operative swelling was noted in more than a third of the rhBMP-2 graft group.[16] Similar post-operative swelling was reported by Shah, Smyth, and Woo[15] in a case report of an rhBMP-2/ACS implant for a cranial vault repair in a 2 year-old boy with craniosynostosis. The patient exhibited severe facial, periorbital, and scalp swelling, resulting in surgical removal of the implant.[15] It has been suggested that the side effect profile of rhBMP-2 is more extreme and more common after such off-label uses.[9] Moreover, the adverse side effects associated with the growth factor are of extreme concern in a pediatric, growing population.

Off-label use of rhBMP-2 is also common in the field of periodontics, where the growth factor is delivered in alveolar defect repair, as well as sinus elevation procedures, by combining pieces of rhBMP-2-soaked ACSs with particulate allograft or xenograft bone materials.[17] Due to

the off-label nature of these uses, there are few clinical studies evaluating their efficacy. A 2012 study evaluated the use of rhBMP-2/ACS in combination with a bovine-derived xenograft in human subjects undergoing sinus elevation procedures.[17] The authors illustrated that in samples in which rhBMP-2/ACS was delivered in combination with a xenograft, there was increased resorption of the graft material, combined with decreased new bone formation, and increased marrow spaces noted histologically, compared to patients treated with the xenograft alone. They concluded that the combination of these materials was not efficacious.[17] Their findings are in contrast with previous work comparing rhBMP-2/ACS with the use of a collagen sponge alone for a similar procedure, in which investigators concluded that the combination of rhBMP-2 and an ACS was the optimal treatment condition.[18] Inconclusive results and limited studies suggest a need for further investigation in this arena.

In light of the aforementioned bioactivity and side effects, as well as the growth factor's often inconsistent clinical performance, much of the scientific and medical community agree that the application of rhBMP-2 for clinical procedures needs further optimization.[11] Hence, with a particular focus on animal studies, there has been a great deal of investigation evaluating the growth factor and its optimal delivery methods.

BMP delivery: Requirements for delivery systems

In vitro studies have demonstrated the efficacy of BMPs in the initiation of osteogenetic pathways, as well as subsequent activation and differentiation of mesenchymal cells.[4] However, in vivo, there are often mixed results; as the protein can be degraded and tissue regeneration is inconsistent.[19] Several factors effect BMP function in vivo. The osteoinductive protein must

remain at the defect site for time enough to allow for the migration of tissue forming cells to the site, and their subsequent proliferation and differentiation.[2] The mode of delivery of BMP is critical for its action. Thus, there has been extensive investigation into the optimal delivery system and carrier for these proteins to the site of bone regeneration.

It is widely agreed that delivery systems for rhBMP-2 should be relatively easy and cost-effective to manufacture, with the ability to be sterilized for in vivo use.[20] The delivery system should be reliable and consistent in form, yet also malleable—with the capacity to be shaped to fit a wide variety of craniofacial defects.[6] They should be porous, allowing the infiltration of mesenchymal cells and blood vessels; yet they should also be mechanically stable, with the ability to withstand compressive and tensile forces.[20] The rate of resorption of the carrier itself should aim to mirror the rate of formation of the new bony tissue. In addition, the rate of osseoregeneration should not be effected by inflammatory or foreign material reactions to the carrier substance itself.[12]

The biologic action of BMPs can be shaped by their carrier and the manner in which they are delivered. The carrier has the ability to alter the local retention of the protein, the release kinetics and mechanism of release, and the overall dose of the protein necessary for osteoinduction and bone formation.[2]

Site of action:

Most critically, the carrier should stabilize the protein, retaining it in place at the site of intended bone formation. If delivered systemically, rhBMP-2 is rapidly cleared from the bloodstream; therefore, delivery of BMP directly to the site of desired bone formation, and in a

carrier that maintains adequate protein levels, is imperative.[2] Early studies illustrated that a significantly larger percentage of the delivered dose of BMP is retained at the delivery site when the protein is administered in a gelatin or collagen-like substance as compared to in a purely soluble form.[21] By retaining the protein at the delivery site, the pharmacokinetics of BMPs can be altered: Chen and Mooney[7] note that delivery methods successful in sequestering growth factors at the site of action result in enhanced bone formation and require an overall lower dose of the growth factor.

rhBMP-2 Release kinetics:

Much investigation has looked into the optimal release kinetic profile for rhBMP-2; yet there does not appear to be a clear consensus as to one particular profile that consistently leads to enhanced bone formation. Studies have indicated that the extremes of protein release are not ideal: i.e. bolus delivery or incredibly low level, slow release do not improve results.[20] In fact, much work has suggested that combining an initial burst release—in order to bring in necessary cell mediators and trigger vasculogenesis—with a slower, long-term delivery of the growth factor can lead to increased osteogenesis.[22]

Determining an ideal release kinetic profile is further complicated by the fact that in vivo factors can alter the functioning the growth factor and its delivery system. Release kinetics are affected by the anatomical site of application. As Geiger et al.[20] suggest, when rhBMP-2 is delivered to a less vascularized area, diffusion of the protein away from that area might succeed the body's ability to bring in the cytokines and cells necessary to commence bone formation. Thus, such situations would benefit from slower, more sustained release of the growth factor.

Different animal species — with variations in size and metabolism — might also require different release profiles of protein to best enhance bone formation.[6]

Release kinetics are difficult to monitor, particularly in vivo. When delivered, the growth factors are subject to several biochemical factors that are not necessarily present in vitro. For instance, bodily fluids, local proteins and enzymes, temperature, and pH can all affect the kinetics of release of rhBMP-2, as well as its subsequent bioactivity.[2]

Protein dosage/concentration:

As suggested above, due to the inconsistent release profiles of rhBMPs, when used clinically, they are often delivered in supraphysiologic doses. This ensures that a critical threshold of protein is isolated at the implant site and is maintained there for a sufficient period of time — this is, after all, the main role of growth factor delivery systems.[2] Delivery systems that have the capacity to control protein release have the potential to decrease the overall dose of BMP necessary for bone regeneration at a defect site.[10] Studies have indicated that by implementing a long-term, slow release delivery system, therapeutic results are enhanced, compared to a similar dose administered via short-term delivery.[10, 23] A lower dose of BMP would lead to not only decreased overall cost, but also potentially fewer adverse side effects from the delivery of excessive amounts of the growth factor.

Delivery systems for rhBMP-2:

With the aforementioned attributes as guidelines, many attempts have been made to engineer delivery systems for the optimal, sustained-release delivery of rhBMP-2. In this

section, we briefly review of some of the material types and attempts that have been made for the sustained delivery of rhBMP-2. There are four major categories of materials that have been examined for their use in drug delivery, each with their own associated advantages and disadvantages.[2, 4, 6] They include natural polymers, inorganic materials, synthetic polymers, and composites of these.

1) Natural polymers:

This category includes materials such as collagen (sponges, gels, demineralized bone matrix, or films), fibrin, chitosan, or hyaluronic acid. Due to their animal sources, they tend to be biocompatible and naturally resorbable, and have the potential to naturally bind to certain growth factors.[6] There are, however, potential issues including lack of availability and sourcing, difficulty in sterilization, and the potential for disease transmission.[24] Several studies have investigated the use of such materials in the delivery of rhBMP-2.[19-21]

As suggested above, it has been suggested that collagen sponges are effective at sequestering rhBMP-2 at the defect site. A 1998 study conducted by Hollinger et al.[21] compared various treatment modalities for critical sized defects in the radii of rabbits: autograft, collagen sponge alone, rhBMP-2 soaked collagen sponge, or no treatment. They concluded that delivering rhBMP-2 with a collagen sponge produced indistinct results from the autograft group, and improved results compared to collagen alone or no treatment at all. The study utilized a large, 35 ug, dose of rhBMP-2 for the defect repair.[21] A review of the use of collagen sponges for the delivery of rhBMP-2 references several similar studies that conclude that rhBMP-2 delivered in a collagen carrier can be efficacious in bone formation.[20] The authors note that

collagen sponges have the ability to elevate local levels of the protein at the defect site, compared to rhBMP-2 delivered alone in a liquid buffer. Yet it is noted that estimates illustrate that 30% of the protein delivered in such collagen sponges is lost shortly after implantation.[20] Another group of investigators concluded that a gel combining heparin and chitosan for rhBMP-2 delivery in intramuscular injections in rats resulted in improved bone formation, when compared to rhBMP-2 implanted with type I collagen.[19]

2) Inorganic materials:

Inorganic materials, such as calcium phosphate cements and ceramics, tend to have the structure most similar to that of bone.[6, 24] Examples of such materials that have been used for rhBMP-2 carriers include hydroxyapatite (HAP) and beta tricalcium phosphate (β-TCP). As they have a bone-like structure, they tend to be osteoconductive, providing a scaffold upon which bone formation can occur. However, this structure can also render them brittle, with a limited potential for malleability.[6] Porous HAP alone as a carrier for rhBMP-2 has illustrated limited potential for bone induction; partially, because the HAP itself does not resorb in a timely fashion.[25]

With numerous advantages, β-TCP is promising for its use in the delivery of rhBMP-2. The ceramic material can be fabricated into three-dimensional scaffolds to fit defect sites; and its porous structure allows for the introduction of cells or other tissue-modifying factors.[26, 27] In a study evaluating HAP versus β-TCP for the delivery of BMP-2, the β-TCP/BMP-2 system proved more effective at bone induction over a range of protein doses in a rat model. Moreover, the authors noted that the retention of BMP-2 inside the scaffold was enhanced in the β-TCP

group compared to HAP/BMP-2 samples, suggesting an improved slow release delivery with the use of β-TCP.[28]

3) Synthetic materials:

Unlike natural polymers, synthetic polymers do not require purification and do not pose a threat of disease transmission. The most widely investigated material in this group are the poly(α-hydroxy acids), a class of synthetics composed of several polymers including poly(lactide) (PLA), poly(glycolide) (PLG), and the copolymer of these two monomers, poly(DL-lactide-co-glycolide) (PLGA). Poly(α-hydroxy acids) are approved by the FDA, and are a common component of resorbable surgical sutures.[29] Via hydrolysis, the polymers degrade into the component monomers. Through various modes of processing, these synthetic materials can be formed into filaments, scaffolds, or microcapsules into which a growth factor, such as BMP-2, can be incorporated.[10, 24, 30] RhBMP-2 is then released by diffusion through the polymer, or by polymer degradation;[7] thus, providing slow, controlled growth factor release. The greatest disadvantage of this group lies in the acidic byproducts released after hydrolysis. The resultant decrease in pH has the potential to alter normal wound healing; and if the byproducts are not efficiently cleared, an inflammatory response can result.[6]

Poly(α-hydroxy acids) have been implicated for the the sustained delivery of BMP-2 and other growth factors.[10, 31, 32] A 2010 study conducted by La et al.[10] compared short-term versus long-term delivery of BMP-2 in mouse clavarial defects over a range of doses from 0 ug to 3 ug. In the short term delivery group, BMP-2 was suspended in a fibrin gel, while the long-term delivery system consisted of heparin-conjugated PLGA nanospheres suspended in a fibrin gel

that was loaded with BMP-2. Electrostatic interactions between BMP-2 and heparin helped to maintain a local concentration of growth factor as well as control its slow release. The authors concluded that long term delivery, with the incorporation of heparin-conjugated PLGA nanospheres, resulted in enhanced ossification at a lower dose of BMP-2 compared to the short-term delivery samples.[10] This study, among others,[10, 31, 32] illustrates the successful delivery of BMP-2 at a sustained rate, utilizing a PLGA carrier.

4) Composites:

Lastly, it has been the aim of some to attempt to combine components from the above groups in order to harness the advantages brought about by each material alone.

In the current paper, we discuss the delivery of rhBMP-2 in PLGA microcapsules. Microcapsules have the ability to provide sustained, slow release of proteins, growth factors, or drugs.[7, 33] The goal of our work is to investigate a slow release delivery of the growth factor, with the hope of limiting the aforementioned side effect profile of rhBMP-2, and improving BMP-derived bone formation. Previous work has concluded that rhBMP-2, encapsulated in PLGA microspheres, is efficacious for the sustained delivery of the protein.[23, 32-34] In particular, work from our laboratory has evaluated the use of rhBMP-2/PLGA microcapsules in two in vivo models. Wink et al.[23] compared the delivery of rhBMP-2/PLGA microspheres and free rhBMP-2 in subcutaneous injection implants in mice. It was concluded that the sustained delivery of rhBMP-2 with PLGA microspheres reduced the effective dose of the growth factor required for ectopic ossification. In a subsequent experiment, the same rhBMP-2/PLGA microcapsules were

investigated for the repair of a critical sized cranial defect in a rabbit model.[35] Treatment groups consisted of no implant for repair, an empty collagen scaffold, a collagen scaffold loaded with free rhBMP-2, or a collagen scaffold loaded with rhBMP-2/PLGA microcapsules. Again, the authors were able to conclude that the delivery of rhBMP-2/PLGA microcapsules resulted in improved bone formation, with greater volume and surface area, and effectively lowered the dose of rhBMP-2 required for bone formation, compared to other groups.[35]

The subject of protein encapsulation for the application or drug or growth factor delivery has been an area of extensive investigation. Typically, encapsulation occurs through the formation of emulsions. An emulsion results from the combination of two or more immiscible liquids that, due to their hydrophobic properties, create small, individual droplets.[36] Emulsions can be oil-in-water (o/w), water-in-oil (w/o), or a combination of the above. In the case of PLGA microcapsules, a water in oil in water (w/o/w) double emulsion is created to encapsulate rhBMP-2 in a PLGA shell.[36] Conventionally, as in the studies described above, proteins have been encapsulated for delivery using a solvent extraction/evaporation method to create the double emulsion, resulting in a poly-disperse microcapsule sample.[34, 37] Additionally, when proteins are encapsulated in such a manner, protein can capsulation efficiency is low, with often excessive protein loss.[38, 39]

Attempts have been made to improve upon the conventional encapsulation method, particularly by the application of microfluidics.[23, 36, 40] Microfluidics involves the generation of one microcapsule at a time using a glass capillary device, thus producing monodisperse particles similar in size and shape, each fabricated in an identical fashion.[36, 41, 42] The emulsions are created when a hydrophobic inner phase, containing a protein for encapsulation, is combined

with a hydrophobic middle phase (PLGA), all within a hydrophilic outer phase. When the solvent from the middle phase evaporates, monodisperse microcapsules result.[41] When proteins are encapsulated, there is virtually no loss of the inner layer protein.[36, 43]

Scaffolds for rhBMP-2 delivery:

As noted above, ideal bone graft materials are malleable and have the ability to maintain the shape of the defect site. For this reason, scaffold apparatuses are a key component in bone tissue engineering — providing an osteoconductive component. Such materials provide an artificial matrix around which bone formation can occur.[26, 44] Scaffolds not only provide a physical framework, but also encourage the proliferation and differentiation of tissue-forming cells; therefore, they are typically porous with a rough surface in order to encourage cell attachment.[26] Three-dimensional pores throughout a scaffold allow for blood vessel infiltration, and the subsequent ingrown of new cells, nutrients, and tissues.[45] Sharaf et al.[46] describe the application of 3D printed poly(ε-caprolactone)(PCL) and beta tricalcium phosphate (β-TCP) scaffolds for their use in bone tissue engineering. In an in vitro study, they concluded that PCL/ β-TCP scaffolds with smaller pore sizes produced increased infiltration and proliferation of bone marrow-derived progenitor cells, and enhanced new collagenous tissue formation.[46]

Conclusion

The application of rhBMP-2 for bone tissue engineering holds great promise for researchers and clinicians in both the medical and dental fields. It has clearly been demonstrated that the sustained, long-term release of the proteins can lead to improved ossification, owing

particularly to the rapid metabolism of the biologically active growth factor when it is delivered alone in solution. Clinically, however, extreme, supraphysiologic doses of the protein are still being delivered to patients regularly, leading to instances of erratic, ectopic bone formation, and even life-threatening side effects. Delivery systems for rhBMP-2 have been investigated extensively; yet still, further exploration into the best means of protein delivery — in order to curb the side effect profile and improve the quality of the bone generated — is warranted.

References

1. Desilets CP, Marden LJ, Patterson AL, Hollinger JO. Development of synthetic bone-repair materials for craniofacial reconstruction. J Craniofac Surg. 1990;1(3):150-3.
2. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: Current challenges in BMP delivery. Biotechnol Lett. 2009;31(12):1817-24.
3. Deatherage J. Bone materials available for alveolar grafting. Oral Maxillofac Surg Clin North Am. 2010;22(3):347-52, v.
4. Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). J Tissue Eng Regen Med. 2008;2(1): 1-13.
5. Carreira AC, Lojudice FH, Halcsik E, Navarro RD, Sogayar MC, Granjeiro JM. Bone morphogenetic proteins: facts, challenges, and future perspectives. J Dent Res. 2014;93(4): 335-45.
6. Li RH, Wozney JM. Delivering on the promise of bone morphogenetic proteins. Trends Biotechnol. 2001;19(7):255-65.
7. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. Pharm Res. 2003;20(8):1103-12.
8. Garrett MP, Kakarla UK, Porter RW, Sonntag VK. Formation of painful seroma and edema after the use of recombinant human bone morphogenetic protein-2 in posterolateral lumbar spine fusions. Neurosurgery. 2010;66(6):1044-9; discussion 9.
9. Woo EJ. Adverse events after recombinant human BMP2 in nonspinal orthopaedic procedures. Clin Orthop Relat Res. 2013;471(5):1707-11.
10. La WG, Kang SW, Yang HS, Bhang SH, Lee SH, Park JH, et al. The efficacy of bone morphogenetic protein-2 depends on its mode of delivery. Artif Organs. 2010;34(12):1150-3.
11. McKay B. Local sustained delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). Conf Proc IEEE Eng Med Biol Soc. 2009;2009:236-7.
12. Schmidmaier G, Schwabe P, Strobel C, Wildemann B. Carrier systems and application of growth factors in orthopaedics. Injury. 2008;39 Suppl 2:S37-43.
13. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: Delivery systems for BMPs in orthopaedic and craniofacial tissue engineering. Biotechnol Lett. 2009;31(12):1825-35.
14. Cahill KS, Chi JH, Day A, Claus EB. Prevalence, complications, and hospital charges associated with use of bone-morphogenetic proteins in spinal fusion procedures. JAMA. 2009;302(1):58-66.
15. Shah MM, Smyth MD, Woo AS. Adverse facial edema associated with off-label use of recombinant human bone morphogenetic protein-2 in cranial reconstruction for craniosynostosis. Case report. J Neurosurg Pediatr. 2008;1(3):255-7.

16. Alonso N, Tanikawa DY, Freitas Rda S, Canan L, Jr., Ozawa TO, Rocha DL. Evaluation of maxillary alveolar reconstruction using a resorbable collagen sponge with recombinant human bone morphogenetic protein-2 in cleft lip and palate patients. Tissue Eng Part C Methods. 2010;16(5):1183-9.
17. Kao DW, Kubota A, Nevins M, Fiorellini JP. The negative effect of combining rhBMP-2 and Bio-Oss on bone formation for maxillary sinus augmentation. Int J Periodontics Restorative Dent. 2012;32(1):61-7.
18. Fiorellini JP, Howell TH, Cochran D, Malmquist J, Lilly LC, Spagnoli D, et al. Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. J Periodontol. 2005;76(4):605-13.
19. Engstrand T, Veltheim R, Arnander C, Docherty-Skogh AC, Westermark A, Ohlsson C, et al. A novel biodegradable delivery system for bone morphogenetic protein-2. Plast Reconstr Surg. 2008;121(6):1920-8.
20. Geiger M, Li RH, Friess W. Collagen sponges for bone regeneration with rhBMP-2. Adv Drug Deliv Rev. 2003;55(12):1613-29.
21. Hollinger JO, Schmitt JM, Buck DC, Shannon R, Joh SP, Zegzula HD, et al. Recombinant human bone morphogenetic protein-2 and collagen for bone regeneration. J Biomed Mater Res. 1998;43(4):356-64.
22. Li B, Yoshii T, Hafeman AE, Nyman JS, Wenke JC, Guelcher SA. The effects of rhBMP-2 released from biodegradable polyurethane/microsphere composite scaffolds on new bone formation in rat femora. Biomaterials. 2009;30(35):6768-79.
23. Wink JD. Regenerating Bone in the Craniofacial Skeleton: Application of PLGA Microspheres for Recombinant Human Bone Morphogenetic Protein 2 Delivery. Philadelphia, PA: University of Pennsylvania; 2014.
24. Seeherman H, Wozney JM. Delivery of bone morphogenetic proteins for orthopedic tissue regeneration. Cytokine Growth Factor Rev. 2005;16(3):329-45.
25. Noshi T, Yoshikawa T, Dohi Y, Ikeuchi M, Horiuchi K, Ichijima K, et al. Recombinant human bone morphogenetic protein-2 potentiates the in vivo osteogenic ability of marrow/hydroxyapatite composites. Artif Organs. 2001;25(3):201-8.
26. Dorozhkin SV. Bioceramics of calcium orthophosphates. Biomaterials. 2010;31(7):1465-85.
27. Bose S, Tarafder S. Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. Acta Biomater. 2012;8(4):1401-21.
28. Tazaki J, Murata M, Akazawa T, Yamamoto M, Ito K, Arisue M, et al. BMP-2 release and dose-response studies in hydroxyapatite and beta-tricalcium phosphate. Biomed Mater Eng. 2009;19(2-3):141-6.
29. Saito N, Murakami N, Takahashi J, Horiuchi H, Ota H, Kato H, et al. Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins. Adv Drug Deliv Rev. 2005;57(7):1037-48.

30. Gentile P, Chiono V, Carmagnola I, Hatton PV. An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. Int J Mol Sci. 2014;15(3):3640-59.
31. Lim TY, Poh CK, Wang W. Poly (lactic-co-glycolic acid) as a controlled release delivery device. J Mater Sci Mater Med. 2009;20(8):1669-75.
32. Kirby GT, White LJ, Rahman CV, Cox HC, Qutachi O, Rose FR, et al. PLGA-Based Microparticles for the Sustained Release of BMP-2. Polymers. 2011;3:571-86.
33. Isobe M, Yamazaki Y, Mori M, Ishihara K, Nakabayashi N, Amagasa T. The role of recombinant human bone morphogenetic protein-2 in PLGA capsules at an extraskeletal site of the rat. J Biomed Mater Res. 1999;45(1):36-41.
34. Isobe M, Yamazaki Y, Oida S, Ishihara K, Nakabayashi N, Amagasa T. Bone morphogenetic protein encapsulated with a biodegradable and biocompatible polymer. J Biomed Mater Res. 1996;32:433-8.
35. Wink JD, Gerety PA, Sherif RD, Lim Y, Clarke NA, Rajapakse CS, et al. Sustained delivery of rhBMP-2 by means of poly(lactic-co-glycolic acid) microspheres: cranial bone regeneration without heterotopic ossification or craniosynostosis. Plast Reconstr Surg. 2014;134(1):51-9.
36. Shah RK, et al. Designer Emulsions Using Microfluidics. Materials Today. 2008;11:18-27.
37. Borselli C, Ungaro F, Oliviero O, d'Angelo I, Quaglia F, La Rotonda MI, et al. Bioactivation of collagen matrices through sustained VEGF release from PLGA microspheres. J Biomed Mater Res A. 2010;92(1):94-102.
38. Nihant N, Schugens C, Grandfils C, Jerome R, Teyssie P. Polylactide microparticles prepared by double emulsion/evaporation technique. I. Effect of primary emulsion stability. Pharm Res. 1994;11(10):1479-84.
39. Yeo Y, Park K. Control of encapsulation efficiency and initial burst in polymeric microparticle systems. Arch Pharm Res. 2004;27(1):1-12.
40. Lee M, Lee EY, Lee D, Park BJ. Stabilization and fabrication of microbubbles: applications for medical purposes and functional materials. Soft Matter. 2015;11(11):2067-79.
41. Utada AS, Lorenceau E, Link DR, Kaplan PD, Stone HA, Weitz DA. Monodisperse double emulsions generated from a microcapillary device. Science. 2005;308(5721):537-41.
42. Xu S, Nie Z, Seo M, Lewis P, Kumacheva E, Stone HA, et al. Generation of monodisperse particles by using microfluidics: control over size, shape, and composition. Angew Chem Int Ed Engl. 2005;44(5):724-8.
43. Pessi J, Santos HA, Miroshnyk I, JoukoYliruusi, Weitz DA, Mirza S. Microfluidics-assisted engineering of polymeric microcapsules with high encapsulation efficiency for protein drug delivery. Int J Pharm. 2014;472(1-2):82-7.
44. Hadlock TA, Vacanti JP, Cheney ML. Tissue engineering in facial plastic and reconstructive surgery. Facial Plast Surg. 1998;14(3):197-203.
45. Lovett M, Lee K, Edwards A, Kaplan DL. Vascularization strategies for tissue engineering. Tissue Eng Part B Rev. 2009;15(3):353-70.

46. Sharaf B, Faris CB, Abukawa H, Susarla SM, Vacanti JP, Kaban LB, et al. Three-dimensionally printed polycaprolactone and beta-tricalcium phosphate scaffolds for bone tissue engineering: an in vitro study. J Oral Maxillofac Surg. 2012;70(3):647-56.