Review Article

Recent Progress in the Construction of Functional Artificial Bone by Cytokine-Controlled Strategies

Xiao-Gang Bao[1], Meng-Chao Shi[2], Chun-Lin Hou[1], Guo-Hua Xu[1]

[1]Department of Orthopedic Surgery, The Spine Surgical Center, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[2]Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland 4059, Australia

Abstract

Objective: Combining artificial scaffolds with stimulatory factors to reconstruct lost bone tissues is one of the hottest research directions. The purpose of this review was to conduct a retrospective survey on the latest reports on artificial bone fabrication with functional cytokines.
Data Sources: The status of related scientific research from the year 2005 to 2018 was analyzed through the mode of literature retrieval in PubMed and VIP Database. The retrieval words are as follows: "bone tissue engineering," "angiogenesis," "cytokines," "osteogenesis," "biomimetic bone marrow," "sol-gel," "delivery system," and the corresponding Chinese words.
Study Selection: After reading through the title and abstract for early screening, the full text of relevant studies was evaluated and those not related with this review had been ruled out.
Results: According to the literature retrospective survey, there were three key points for the successful construction of functional artificial bones: (1) the continuous supply of relatively low concentration of cytokines during the required period; (2) the delivery of two or more cytokines essential to the process and ensure the relatively spatial independence to reduce the unnecessary interference; and (3) supporting the early-stage angiogenesis and late-stage osteogenesis, respectively, regulating and balancing the crosslinking of both to avoid the surface ossification that would probably block the osteogenesis inside.
Conclusions: The synergistic effect of both angiogenic factors and osteogenic factors applied in bone regeneration is a key point in the combined functional artificial bone. Through analysis, comparison, and summary of the current strategies, we proposed that the most promising one is to mimic the natural bone marrow function to facilitate the regeneration process and ensure the efficient repair of large weight-bearing bone defect.

Key words: Angiogenesis; Cytokines; Functional Artificial Bone; Osteogenesis; Spatiotemporal Delivery

Introduction

With the artificial bone implantation being one of the most promising approaches to repair large bone defect, the rapid transfer and self-reconstruction of artificial bone toward autogenous bone is the only access to solve the bone fracture, collapse, nonunion, and possible movement.[1] As indicated in previous studies, the insufficient supply of blood, nutrition, and cell migration toward the center will impede the osteogenesis and restrict new bone formation thoroughly.[2] It has caused the high rate of 60% fracture in patients during the past 10 years.[3] The purpose of this study was to summarize the construction of functional artificial bones, including the pathophysiological mechanism of bionic autologous bone regeneration, and suggest how to regulate cytokines in accordance with the natural operation rules of bone or vascular regeneration. The strategies provide a promising prospect for the early-stage rapid vascularization of large segment of artificial bone and the ultimate omnidirectional stereoscopic osteogenesis effect.[4]

Importance of Long-Term and Sequential Release of Cytokines

The sequential and synergic role of a series of angiogenic factors in the process of early-stage vascularization plays

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.244105

Address for correspondence: Dr. Guo-Hua Xu, Department of Orthopedic Surgery, The Spine Surgical Center, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
E-Mail: xuguohuamail@smmu.edu.cn

Received: 02-07-2018 **Edited by:** Yi Cui
How to cite this article: Bao XG, Shi MC, Hou CL, Xu GH. Recent Progress in the Construction of Functional Artificial Bone by Cytokine-Controlled Strategies. Chin Med J 2018;131:2599-604.

a key role. To meet this urgent requirement, it is essential to find the solution that could maintain a locally long-term and sequential release of these factors. It has been revealed that vascular ingrowth in long bone implant mainly relies on the contact between the implant and the surrounding tissue in "parent tube" sprout.[5] In general, the effective nutrient exchange of cells in the artificial scaffolds only occurs within the distance of 200 μm,[6] and the effective distance from osteocytes to the surrounding vessels is less than 100 μm.[7] In present clinical and experimental studies, relative slow and immature angiogenesis was only observed on the interface between artificial bone and host bone tissue, which usually led to the insufficient oxygen and nutrient supply into the inner part and further resulted in cell poor growth or apoptosis.[8] This could hinder the degradation of artificial bone and the reconstruction of new bone.[9] Recent studies on the application of variety of stimulatory cytokines have made progress and proved the positive effects of the combination of angiogenic and osteogenic factors.[10]

Mature and functional angiogenesis is a process in which multiple cytokines synergistically and sequentially play a key role in different stages [Figure 1].[11] Among them, vascular endothelial growth factor (VEGF) family is the most specific promoter and the most important activator, which is widely used to promote the vascularization of artificial bone scaffolds.[12] Combining VEGF with different artificial bone scaffolds can effectively promote capillary growth, increase vascular density, stimulate rapid formation of new blood vessels, and form high-density blood vessels anastomosing with host blood vessels.[13,14] VEGF, along with platelet-derived growth factors (PDGFs), has specific affinity for vascular cell recruitment and gathering surrounding the new vessels to provide structural support and stimulate angiogenesis.[15] The properties and characteristics of PDGF and VEGF are continuous and sequential at different stages of angiogenesis.[16] VEGF is also closely related to bone growth factors (e.g., Bone Morphogenetic protein, BMP), showing synergistic stimulation in building the signaling pathway of bone and vascular genesis.[17] Compared to the single use of VEGF, Combined application of BMP-2 and VEGF indicated a synergic stimulation and could improve vascularization of artificial bone increasing the vascular density, and further accelerate the healing of artificial bone.[18] These factors are essential in the angiogenesis and its stabilization during different stages.[19] They are independent respectively and could show great potential via combination with each other under certain control on the temporal and spatial release kinetics.[20]

At present, there are many studies on the multifactor synergistic effects of osteogenesis and angiogenesis. However, few of them focused on the sequential release of multiple factors in different space-time patterns and revealed underlying mechanism in promoting the vascularization and osteogenesis of large bone graft in depth.[21] How to construct the stable system of multifactors within the artificial bone scaffolds? How to manage and control the long-term release of these factors? And how to avoid the enzyme consumption and maintain a high local target rate? All the above are the bottleneck problems faced by artificial bone construction and new strategies are needed urgently.[22]

Combination Strategies for Sustainable Long-Term Delivery of Cytokines

The application of biodegradable polymers in cytokine delivery is one of the best options to maintain the viability of factors and achieve sustainable long-term release. However, it still remains a big challenge to avoid the burst release, to maintain a longer period, and to overcome the difficulties in their combination with scaffolds. In order to avoid the rapid release and enzyme consumption, and to ensure the relative independence and long-term activity of each factor locally, biodegradable polymer materials are seen as an effective drug cargo.[23] It can sustain a general long period of cytokine release and remain an abundant concentration at specific spot.[4,15]

Encapsulation of various factors has shown great potential for the independent release of multiple cytokines in the same artificial bone.[24] Most of the previous reports which focused on the prolong of release period for osteogenic and angiogenic stimulation have shown poor stability on the loading efficiency of cytokines in the artificial bone.[25] Furthermore,

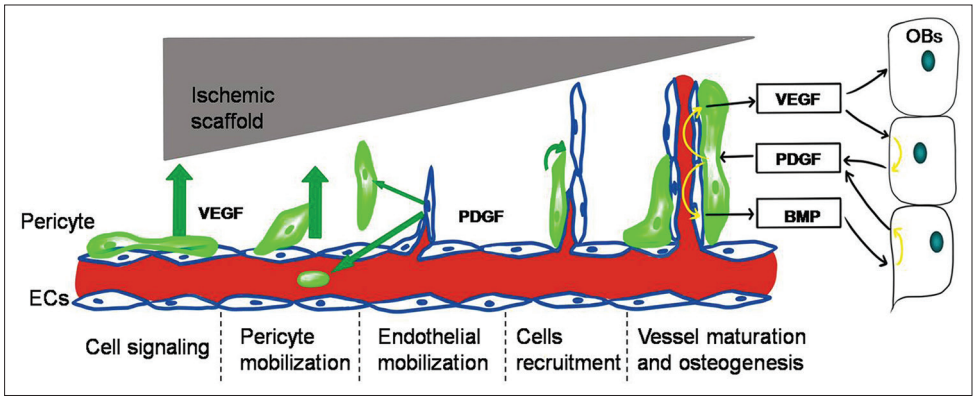

Figure 1: Schematic diagram of the role of VEGF, PDGF, and BMP at different stages in bone regeneration. All having similar functional mechanism and interacting with each other in bone formation. EC: Edndothelial cell; OB: Osteoblast; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; BMP: Bone morphogenetic protein.

the mismatching of release time and the regenerative process is another challenge.[26] Although several studies indicated that the application of multilayer barriers with microspheres as delivery cargo could effectively extend the period,[27,28] it failed in achieving the coordination between cytokine releasing and new bone formation process.[29] It is essential to realize the ideal early-stage rapid angiogenesis and late-stage ultimate omnidirectional osteogenesis of artificial bone.[30]

Thermosensitive hydrogels, which serve as three-dimensional (3D) matrix, can be combined with the factor-loaded microspheres to form a "core-shell" system. With the degradation property of specific hydrogels and the microscale carrier, cytokines could be released at different time points with controllable spatial pattern. It is likely to avoid burst release at first place and prolong the release period. Using the phase-change properties of thermosensitive hydrogel, the stable combination of artificial bone and cytokine-loaded system can be achieved. This may further solve the insufficient inside angiogenesis and new bone formation caused by surface ossification.

Controllable Spatial and Sequential Release of Multiple Cytokines for Synchronous New Bone Formation

Due to the satisfactory biocompatibility, the 3D microstructure, and similarity of extracellular matrix, hydrogel has been widely studied in bone tissue engineering.[31] Seed cells such as vascular endothelial cells can adhere, migrate, and proliferate in the 3D hydrogel scaffolds.[32] Further reports have indicated that the composition of certain angiogenic cytokines in chitosan hydrogel, hyaluronic acid hydrogel, gelatin, and methyl acrylate hydrogel could significantly stimulate vascular endothelial cell growth.[33-35] Furthermore, the controllable cytokine release further benefits the migration and proliferation of vascular endothelial cells.

Recently, Ding's group from Fudan University has developed a novel thermosensitive hydrogel, the PLGA-PEG-PLGA series. The hydrogel possesses adjustable injection property, favorable biodegradability, and good biocompatibility and shows great potential in the prevention of postoperative adhesion, sustained drug release, tissue repair, etc.[36,37] Below the body temperature, the hydrogels are in sol state and can transfer to a semi-solid gel state as the temperature increases [Figure 2]. The characterization of PLGA-PEG-PLGA hydrogel indicated that it was suitable to be used as cytokine-loaded materials to embed into artificial bone [Figure 3].[38] It can mimic the behavior of extracellular matrix to regulate the local cellular response, stimulate tissue repair, and control the release of active proteins.

Furthermore, the hydrogel is used as dual-cytokine carrier for bone repair in our previous studies, which VEGF-165 and BMP-2 could be effectively loaded and sustainably released for a long period of 3 weeks.[38] It is expected that by using

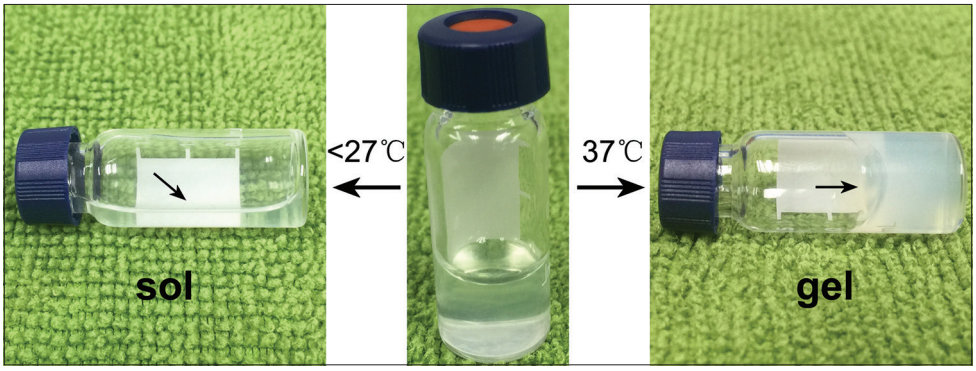

Figure 2: The state transfer of thermosensitive hydrogel PLGA-PEG-PLGA.

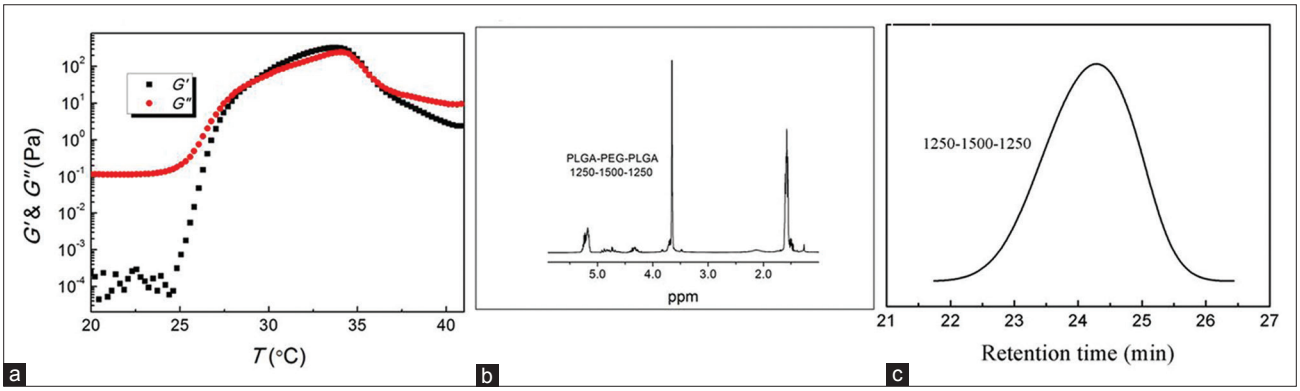

Figure 3: The sol-gel transition process of the copolymer-saline solution (transition temperature of 27°C; a), 1H NMR spectrum of the PLGA-PEG-PLGA copolymer (b), GPC analysis of the copolymer shows a unimodal distribution (c). GPC: Gel permeation chromatography.

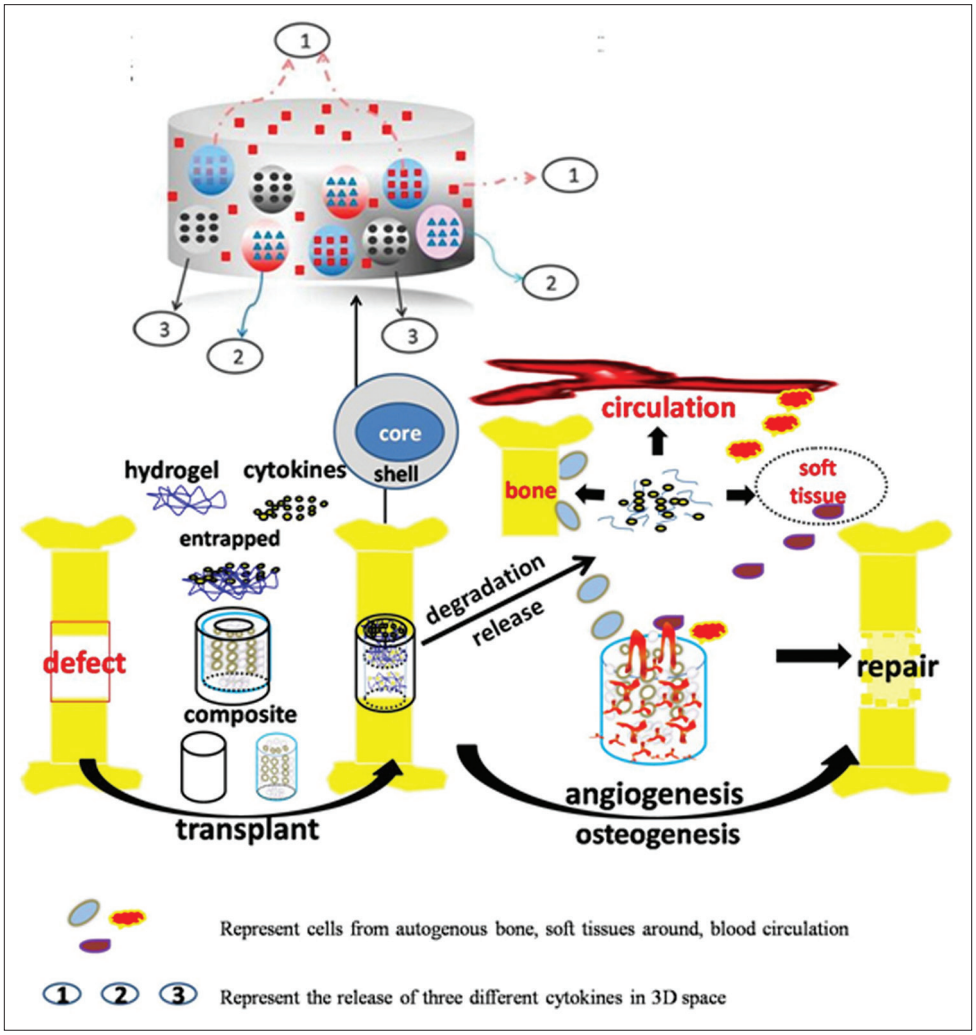

Figure 4: Schematic diagram of functional artificial bone with multicytokine loading and controllable release.

proper microspheres prepared by biocompatible materials such as PLGA, which could be infiltrated into the 3D porous structure of artificial bone scaffold, a multilayer delivery system could be constructed.[39] Multiple cytokines including VEGF-165, PDGF-BB, and BMP-2 could be embedded into artificial bone successfully and stably.[40] Through negative pressure loading process and the following state transfer of the thermosensitive hydrogel under body temperature, the cytokine-loaded microspheres could be effectively combined within the 3D structure. It could mimic the natural function of red bone marrow, supply key cytokines to promote angiogenesis, accelerate material degradation, and stimulate osteogenesis of artificial bone [Figure 4]. This hydrogel-based bioactive system could mimic the effect of red bone marrow in the marrow cavity of cancellous bone, thus endowing the artificial bone with spatial and all-dimensional angioinduction and osteoinduction properties.

Problems and Prospective

To sum up, despite the variety of studies on stimulation of both vascularization and osteogenesis, few has been done on the underlying mechanism of spatial and sequential release of different cytokines. It still remains great challenge in finding the solutions to construct the stable system of multifactors within the artificial bone scaffolds, to manage and control the long-term release of these factors, and to avoid the enzyme consumption and maintain a high local target rate. According to the retrospective literature survey, we summarized that there are three key points for the successful construction of functional artificial bones: (1) the continuous supply of relatively low concentration of cytokines during the required period; (2) the delivery of two or more cytokines essential to the process and ensure the relative spatial independence to reduce the unnecessary interference; and (3) supporting the early-stage vascularization and late-stage osteogenesis, respectively, regulating and balancing the crosslinking of them both to avoid the surface ossification that will block the osteogenesis inside. To address all the essential factors, one of the key challenges is the fabrication of scaffold system. The material choice should be focused on the loading and release properties. With the basic biosafety, biocompatibility, and bioactivity for bone regeneration, the future direction may rely on the introduction of smart control system and multichannel loading system. Using the advanced hydrogel system, which could be easily modified by chemical

reactions, there is a high possibility to create suitable delivery system. Through application of the additive manufacturing technique, such as customized 3D printing, the multichannel or hierarchical structure could be obtained. It is promising to mimic the property of natural red bone marrow which ensures that all the cytokines are filling omnidirectionally in the artificial bone scaffold and released sustainably for a matching period during new bone formation [Figure 3]. This strategy, which is in line with the physiological mechanism of the body, is expected to offer new options to solve the problem on reconstruction and repair of large bone defects.

Conclusion

This review describes the latest researches regarding the construction of functional artificial bone by cytokine-controlled strategies. It still remains great challenge to construct the stable system of multifactors within the artificial bone scaffolds. We proposed the most promising strategy for sufficient repair of large weight-bearing bone defect in this review, that is, to construct the mimetic substitute for natural bone marrow function with proper degradability. Herein, controllable spatial release of cytokines by using specific hydrogels and proper microscale carrier will be a wise choice.

Financial support and sponsorship

This study was supported by grants from the Shanghai Committee of Science and Technology, China (No. 15411951000), and the National Natural Science Foundation of China (No. 81271954).

Conflicts of interest

There are no conflicts of interest.

References

1. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: Classic options, novel strategies, and future directions. J Orthop Surg Res 2014;9:18. doi: 10.1186/1749-799X-9-18.
2. Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, Walocha JA, Niedźwiedzki T. The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis 2017;20:291-302. doi: 10.1007/s10456-017-9541-1.
3. Liu Y, Lim J, Teoh SH. Review: Development of clinically relevant scaffolds for vascularised bone tissue engineering. Biotechnol Adv 2013;31:688-705. doi: 10.1016/j.biotechadv.2012.10.003.
4. Jayaraman P, Gandhimathi C, Venugopal JR, Becker DL, Ramakrishna S, Srinivasan DK, *et al.* Controlled release of drugs in electrosprayed nanoparticles for bone tissue engineering. Adv Drug Deliv Rev 2015;94:77-95. doi: 10.1016/j.addr.2015.09.007.
5. Saran U, Gemini Piperni S, Chatterjee S. Role of angiogenesis in bone repair. Arch Biochem Biophys 2014;561:109-17. doi: 10.1016/j.abb.2014.07.006.
6. Kannan RY, Salacinski HJ, Sales K, Butler P, Seifalian AM. The roles of tissue engineering and vascularisation in the development of micro-vascular networks: A review. Biomaterials 2005;26:1857-75. doi: 10.1016/j.biomaterials.2004.07.006.
7. Mercado-Pagán ÁE, Stahl AM, Shanjani Y, Yang Y. Vascularization in bone tissue engineering constructs. Ann Biomed Eng 2015;43:718-29. doi: 10.1007/s10439-015-1253-3.
8. Santos MI, Reis RL. Vascularization in bone tissue engineering: Physiology, current strategies, major hurdles and future challenges. Macromol Biosci 2010;10:12-27. doi: 10.1002/mabi.200900107.
9. García JR, García AJ. Biomaterial-mediated strategies targeting vascularization for bone repair. Drug Deliv Transl Res 2016;6:77-95. doi: 10.1007/s13346-015-0236-0.
10. Cui Q, Dighe AS, Irvine JN Jr. Combined angiogenic and osteogenic factor delivery for bone regenerative engineering. Curr Pharm Des 2013;19:3374-83. doi: 10.2174/1381612811319190004.
11. Brudno Y, Ennett-Shepard AB, Chen RR, Aizenberg M, Mooney DJ. Enhancing microvascular formation and vessel maturation through temporal control over multiple pro-angiogenic and pro-maturation factors. Biomaterials 2013;34:9201-9. doi: 10.1016/j.biomaterials.2013.08.007.
12. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011;473:298-307. doi: 10.1038/nature10144.
13. Harris GM, Rutledge K, Cheng Q, Blanchette J, Jabbarzadeh E. Strategies to direct angiogenesis within scaffolds for bone tissue engineering. Curr Pharm Des 2013;19:3456-65. doi: 10.2174/1381612811319190011.
14. Wang K, Chen X, Pan Y, Cui Y, Zhou X, Kong D, *et al.* Enhanced vascularization in hybrid PCL/gelatin fibrous scaffolds with sustained release of VEGF. Biomed Res Int 2015;2015:865076. doi: 10.1155/2015/865076.
15. De la Riva B, Sánchez E, Hernández A, Reyes R, Tamimi F, López-Cabarcos E, *et al.* Local controlled release of VEGF and PDGF from a combined brushite-chitosan system enhances bone regeneration. J Control Release 2010;143:45-52. doi: 10.1016/j.jconrel.2009.11.026.
16. Grellier M, Bordenave L, Amédée J. Cell-to-cell communication between osteogenic and endothelial lineages: Implications for tissue engineering. Trends Biotechnol 2009;27:562-71. doi: 10.1016/j.tibtech.2009.07.001.
17. Hu K, Olsen BR. The roles of vascular endothelial growth factor in bone repair and regeneration. Bone 2016;91:30-8. doi: 10.1016/j.bone.2016.06.013.
18. Aryal R, Chen XP, Fang C, Hu YC. Bone morphogenetic protein-2 and vascular endothelial growth factor in bone tissue regeneration: New insight and perspectives. Orthop Surg 2014;6:171-8. doi: 10.1111/os.12112.
19. Spiller KL, Vunjak-Novakovic G. Clinical translation of controlled protein delivery systems for tissue engineering. Drug Deliv Transl Res 2015;5:101-15. doi: 10.1007/s13346-013-0135-1.
20. Samorezov JE, Alsberg E. Spatial regulation of controlled bioactive factor delivery for bone tissue engineering. Adv Drug Deliv Rev 2015;84:45-67. doi: 10.1016/j.addr.2014.11.018.
21. Bayer EA, Gottardi R, Fedorchak MV, Little SR. The scope and sequence of growth factor delivery for vascularized bone tissue regeneration. J Control Release 2015;219:129-40. doi: 10.1016/j.jconrel.2015.08.004.
22. Newman MR, Benoit DS. Local and targeted drug delivery for bone regeneration. Curr Opin Biotechnol 2016;40:125-32. doi: 10.1016/j.copbio.2016.02.029.
23. Ma G. Microencapsulation of protein drugs for drug delivery: Strategy, preparation, and applications. J Control Release 2014;193:324-40. doi: 10.1016/j.jconrel.2014.09.003.
24. Suárez-González D, Lee JS, Diggs A, Lu Y, Nemke B, Markel M, *et al.* Controlled multiple growth factor delivery from bone tissue engineering scaffolds via designed affinity. Tissue Eng Part A 2014;20:2077-87. doi: 10.1089/ten.tea.2013.0358.
25. Yun YR, Jang JH, Jeon E, Kang W, Lee S, Won JE, *et al.* Administration of growth factors for bone regeneration. Regen Med 2012;7:369-85. doi: 10.2217/rme.12.1.
26. Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. Adv Drug Deliv Rev 2012;64:1257-76. doi: 10.1016/j.addr.2012.05.006.
27. Sperling LE, Reis KP, Pranke P, Wendorff JH. Advantages and challenges offered by biofunctional core-shell fiber systems for tissue engineering and drug delivery. Drug Discov Today 2016;21:1243-56. doi: 10.1016/j.drudis.2016.04.024.
28. Perez RA, Kim JH, Buitrago JO, Wall IB, Kim HW. Novel therapeutic core-shell hydrogel scaffolds with sequential delivery of cobalt and

bone morphogenetic protein-2 for synergistic bone regeneration. Acta Biomater 2015;23:295-308. doi: 10.1016/j.actbio.2015.06.002.
29. Santo VE, Gomes ME, Mano JF, Reis RL. Controlled release strategies for bone, cartilage, and osteochondral engineering – Part II: Challenges on the evolution from single to multiple bioactive factor delivery. Tissue Eng Part B Rev 2013;19:327-52. doi: 10.1089/ten.TEB.2012.0727.
30. Dyondi D, Webster TJ, Banerjee R. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. Int J Nanomedicine 2013;8:47-59. doi: 10.2147/IJN.S37953.
31. Kondiah PJ, Choonara YE, Kondiah PP, Marimuthu T, Kumar P, du Toit LC, *et al.* A review of injectable polymeric hydrogel systems for application in bone tissue engineering. Molecules 2016;21. pii: E1580. doi: 10.3390/molecules21111580.
32. Nguyen BB, Moriarty RA, Kamalitdinov T, Etheridge JM, Fisher JP. Collagen hydrogel scaffold promotes mesenchymal stem cell and endothelial cell coculture for bone tissue engineering. J Biomed Mater Res A 2017;105:1123-31. doi: 10.1002/jbm.a.36008.
33. Prakash Parthiban S, Rana D, Jabbari E, Benkirane-Jessel N, Ramalingam M. Covalently immobilized VEGF-mimicking peptide with gelatin methacrylate enhances microvascularization of endothelial cells. Acta Biomater 2017;51:330-40. doi: 10.1016/j.actbio.2017.01.046.
34. Wang LS, Lee F, Lim J, Du C, Wan AC, Lee SS, *et al.* Enzymatic conjugation of a bioactive peptide into an injectable hyaluronic acid-tyramine hydrogel system to promote the formation of functional vasculature. Acta Biomater 2014;10:2539-50. doi: 10.1016/j.actbio.2014.02.022.
35. Ci T, Li T, Chang G, Yu L, Ding J. Simply mixing with poly (ethylene glycol) enhances the fraction of the active chemical form of antitumor drugs of camptothecin family. J Control Release 2013;169:329-35. doi: 10.1016/j.jconrel.2012.12.004.
36. Yu L, Hu HT, Chen L, Bao XG, Li YZ, Chen L, *et al*. Comparative studies of thermogels in preventing post-operative adhesions and corresponding mechanisms. Biomater Sci 2014;2:1100-9. doi: 10.1039/C4BM00029C.
37. Zhang Z, Lai Y, Yu L, Ding J. Effects of immobilizing sites of RGD peptides in amphiphilic block copolymers on efficacy of cell adhesion. Biomaterials 2010;31:7873-82. doi: 10.1016/j.biomaterials.2010.07.014.
38. Bao X, Zhu L, Huang X, Tang D, He D, Shi J, *et al*. 3D biomimetic artificial bone scaffolds with dual-cytokines spatiotemporal delivery for large weight-bearing bone defect repair. Sci Rep 2017;7:7814. doi: 10.1038/s41598-017-08412-0.
39. Fredenberg S, Wahlgren M, Reslow M, Axelsson A. The mechanisms of drug release in poly (lactic-co-glycolic acid)-based drug delivery systems – A review. Int J Pharm 2011;415:34-52. doi: 10.1016/j.ijpharm.2011.05.049.
40. Tarafder S, Koch A, Jun Y, Chou C, Awadallah MR, Lee CH, *et al.* Micro-precise spatiotemporal delivery system embedded in 3D printing for complex tissue regeneration. Biofabrication 2016;8:025003. doi: 10.1088/1758-5090/8/2/025003.

细胞因子控释策略在功能化人工骨构建中的最新进展

摘要

目的：将人工骨支架与具有促进作用的细胞因子相结合，用于修复骨缺损是骨组织工程领域的一大研究热点。本综述通过文献调研，总结该策略目前的最新进展，分析功能因子在功能支架构建中的应用现状。
数据来源：本综述中文献调研时间范围为2005年到2018年，采用文献数据库PubMed和维普数据。采用搜索关键词为：骨组织工程、血管化、细胞因子、成骨化、人工骨髓、溶胶-凝胶、传输体系，包含英文与中文对应词汇。
研究选择：通过阅读题目和摘要进行初步筛选，排除不相关研究，保留相关度较高研究工作并进行全文阅读分析。
结果：根据文献追溯，我们总结出目前细胞因子策略在功能化人工骨成功构建中的三大关键点：1）在要求阶段内细胞因子较低浓度的持续释放；2）两种或以上细胞因子在传输过程中的相对空间独立性及干扰规避；3）支持骨修复过程中的早期血管化和后期骨化，协调平衡二者交互作用，防止表面骨化阻碍内部成骨。
结论：实现促成骨和促成血管因子的协同作用是功能化人工骨促进骨修复的关键。通过对已有文献的总结、比较和分析，我们提出以模拟自然骨髓功能的方式构建因子释放体系，在骨修复领域最具应用前景。