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Bioencapsulation technologies in tissue engineering

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Abstract

Bioencapsulation technologies have played an important role in the developing successes of tissue engineering. Besides offering immunoisolation, they also show promise for cell/tissue banking and the directed differentiation of stem cells, by providing a unique microenvironment. This review describes bioencapsulation technologies and summarizes their recent progress in research into tissue engineering. The review concludes with a brief outlook regarding future research directions in this field.

Keywords

Bioencapsulation; Electrostatic spray; Microcapsules; Microencapsulation; Microfluidics; Tissue engineering

Introduction

Bioencapsulation technology has shown great promise for tissue engineering and cell-based therapies. First, bioencapsulation technology can be applied to cell encapsulation, helping to

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overcome the difficulties associated with immunorejection of transplanted tissues and cells (1–4). Traditional methods to avoid rejection involve use of immunosuppressive drugs that are not ideal for the health of the patient (5). The encapsulation of living cells in macroscale or microscale capsules provides a promising route for immunoisolation; the capsule's membrane protects the encapsulated cells from both the host's immune system and mechanical stresses, while allowing free diffusion of nutrients and metabolic waste to and from the encapsulated cells for their survival (4). Second, bioencapsulation technologies can be used for directed differentiation of stem cells for constructing different tissue types with high efficiency and specificity compared with 2D cell differentiation (6–10). Third, bioencapsulation technologies can be applied to cell cryopreservation to help resolve the issue of tissue preservation before transplantation (4, 11–15). Further promise is demonstrated by the confirmation that biocapsules can be utilized for creating artificial cells (16–18), constructing lung alveolus-like structures and vascularizing 3D tissues (19), which are novel and emerging foci of tissue engineering.

In this review, commonly used bioencapsulation materials and methods are introduced and compared. Particularly, bioencapsulation using cells as novel and potential materials is included. The most recent research and clinical progress in applications of bioencapsulation technologies in tissue engineering have been summarized in various categories. Bioencapsulation in bioprinting and cell/tissue cryopreservation – two emerging fields of tissue engineering – have also been reviewed. Lastly, opinions on challenges and future directions of bioencapsulation in tissue engineering, including scaling-up and vascularized 3D tissue construction, have been provided.

Bioencapsulation materials and methods

Bioencapsulation materials

Both natural and synthetic polymers have been used for bioencapsulation. Natural polymers such as alginate, pectin, agarose, collagen and hyaluronic acid are abundant and biocompatible and can be used for bioencapsulation under mild conditions (20). However, their product quality and characteristics can vary broadly among resources and batches. It is well known that a natural polymer's purity and composition, such as the guluronic and mannuronic acid ratio of alginate, highly influence the capsule's performance (21–23). Synthetic polymers such as poly(ethylene glycol) (PEG), 2-hydroxyethyl methacrylate (HEMA) and poly(lactic-*co*-glycolic acid) (PLGA) exhibit more consistent chemical compositions and molecular weights due to the minimized batch-to-batch variations (4, 23–25). Unfortunately, when using synthetic polymers for bioencapsulation, unfavorable conditions are often inevitable, such as exposure to UV light and nonphysiological pH and/or temperature conditions (25).

Among the natural and synthetic polymers, alginate and PEG are two of the most commonly used bioencapsulation materials. Alginates, anionic biopolymers mainly extracted from seaweed, are linear polysaccharides (26). Alginates are composed of α-L-guluronic acid (G) and β-D-mannuronic acid (M) blocks. Formation of the divalent cation junctions – of GG-GG, MG-GG and MG-MG – between alginate molecules leads to the gelation of alginate (formation of the alginate hydrogel) (4). In general, alginate microcapsules must be coated

with a polycation, such as poly-L-lysine or chitosan, to enhance stability and impart permselectivity and PEG to improve the biocompatibility for tissue-engineering applications (9, 27, 28). Figure 1 depicts typical images of alginate-chitosan-alginate microcapsules for 3D culture of mesenchymal stem cells. Cells maintained high viability in both regular and differentiation culture medium (Fig. 1), and successfully differentiated as directed (6). Alginate also exhibits excellent in vivo stability (29). However, multiple factors can influence alginate-based capsule stability after transplantation, such as the implantation site and capsule composition (30). Retrieval of live encapsulated porcine islets from a patient 9.5 years after xenotransplantation has been reported (31). More importantly, clinical trials of several alginate-based encapsulation systems have been or are being conducted, such as those with GLP-1 CellBeads® (alginate microcapsules containing allogenic mesenchymal cells which are genetically modified to secrete glucagon-like peptide-1 [GLP-1] for the treatment of stroke patients with space-occupying intracerebral hemorrhage; the study has been terminated) (32) and NTCELL® (alginate-encapsulated porcine choroid plexus cells for xenotransplantation in patients with Parkinson's disease; a Phase I/IIa clinical trial was completed in 2015 with promising results, and a Phase IIb study began in 2016) (33).

PEG and its derivatives, e.g., poly(ethylene glycol) diacrylate [PEGDA], have been widely used in tissue engineering due to their biocompatibility and ability to be altered to physically mimic soft tissues (34, 35). PEG is one of the few synthetic polymers that can be used for both microencapsulation and macroencapsulation (36), and it has been extensively studied for the surface modification of scaffolds, such as vascular grafts, due to its nonimmunogenicity and nonantigenicity (37, 38). There are different methods for preparing soft PEG gels, such as crosslinking via copper-free strain azide-alkyne cycloaddition (39) and thiol-ene click chemistry (40). An example of PEG hydrogel microcapsules is illustrated in Figure 2A. The figure shows a phase contrast image of mesenchymal stem cell–loaded PEG hydrogel microcapsules. Microcapsules create uniform surfaces without rough edges. Lathuilère et al (41) showed that myogenic cells encapsulated in a biomimetic PEG-based hydrogel matrix could survive at high density for several months. In addition, a rapamycin-containing PEG coating has been shown to be able to improve the biocompatibility of alginate microcapsules during xenotransplantation (28).

To improve encapsulated cell migration, attachment, proliferation and matrix remodeling, several different approaches have been explored. These include chemical modification of encapsulation materials by cross-linking with Arg-Gly-Asp (RGD; a cell adhesion motif) or gelatin (43), as well as cell encapsulation in core-shell structured capsules (7, 8). As an example, multiple types of cells encapsulated within RGD peptide–modified alginate microcapsules displayed improved cell adhesion and proliferation (44). To generate a liquid core, alginate hydrogel beads first must be coated with poly-L-lysine or chitosan before liquefying the center, which is a complex process (9). One-step fabrication of alginate coreshell microcapsules has been used to encapsulate embryonic stem cells with improved cell proliferation, aggregation and directed differentiation efficiency (7, 8).

Interestingly, cells have also been used as the encapsulation material. For instance, islets have been successfully encapsulated with living cells (HEK 293 cells) through polyDNA-PEG-lipid conjugates (Fig. 2B). The resulting encapsulated islets were found to retain their

function (42, 45). Additionally, immobilization of islets using Sertoli cells for immunoprotection has also been investigated (46).

Bioencapsulation methods

Methods for bioencapsulation including electrostatic spray, microfluidic channel/nozzle, vibration nozzle, laminar jet breakup (JetCutter) and air-jet encapsulation have been developed (47–50). Electrostatic spray and microfluidic channel/nozzle are the most common methods used for bioencapsulation, especially since they both show distinct potential for producing core-shell microcapsules (Fig. 3) (7, 8). The vibration nozzle technique is considered to be the most industrially up-scalable technique for microcapsule production, especially when the viscosity of the encapsulation solution is low (51). Although laminar jet breakup and air-jet encapsulation technologies have high throughput, it is difficult to obtain evenly sized capsules (52, 53).

The electrostatic spray method offers the advantages of cytocompatibility, ease of operation and ability to prepare microcapsules in a sterile environment (4, 50). During the electrostatic spray process, droplets of polymer solution are formed on the tip of the nozzle and sprayed into a gelling bath, such as a divalent cation solution, as a result of the electrostatic force between the gelling bath and the nozzle, the surface tension and gravity (48). When using the microfluidic channel/nozzle approaches, small mono-dispersed microcapsules (<200 μm) can easily be manufactured compared with other methods (54). Flow focusing (with 1 core flow surrounded by a sheath stream) and T-junctions (with 1 core flow and 1 sheath stream crossing at a 90° angle) are 2 common platforms for microfluidic-based encapsulation. Generally, a polymer solution containing cells creates the core flow. This is sheared by the oil (continuous) flow. As a result of the immiscible nature of water and oil, droplets are formed (7, 47). Rapid exchange of the toxic oil phase in a microencapsulation chip is critical to maintaining a high cell survival rate (55). Under optimal conditions, both electrostatic spray and microfluidic channel/nozzle methods have been shown to be safe for cell encapsulation, while producing capsules with uniform sizes. For example, encapsulated mesenchymal stem cells, produced through the electrostatic spray method, have survived (>95% cell viability) and proliferated successfully well within alginate microcapsules during a month-long study period (6). In another study, Agarwal et al (7) encapsulated mouse embryonic stem cells in the liquid core of alginate microcapsules using a microfluidic flow-focusing device. The encapsulated cells were found to survive well (>92% cell viability) and proliferate to form a single aggregate in each microcapsule within 7 days. It is worth mentioning that commercial encapsulators are available, such as the BÜCHI® Labortechnik AG Encapsulator B-390, which is based on the electro-spray-vibration method (56), and Cellena® portable microencapsulation equipment, which uses the flow-focusing technology (57).

Current applications of bioencapsulation technologies in tissue engineering

Bone/cartilage tissue engineering

There is a vast body of work published on bioencapsulation for bone/cartilage tissue engineering. In one significant example, Olabisi et al reported the rapid heterotopic ossification by an intramuscular injection of encapsulated adenovirusesbone morphogenetic protein 2 (AdBMP2)–transduced fibroblasts in PEGDA hydrogels (34). In addition, it was proven that the cryopreservation of microencapsulated BMP2-expressing mesenchymal stem cells did not negatively affect the heterotrophic ossification (Fig. 4) (35). Moreover, biocapsules have been applied to construct scaffolds for bone/cartilage tissue engineering. For instance, a biodegradable PEG-based microcavitary hydrogel for cartilage tissue engineering was developed, where gelatin microspheres were used as a porogen in cell-laden constructs to create microscale cavities (58). Furthermore, for bone tissue-engineering applications, human embryonic stem cell–derived mesenchymal stem cells (hESCd-MSCs) encapsulated in alginate microbeads in macroporous calcium phosphate cement were also tested (59).

Cardiac tissue engineering

Cell encapsulation shows promise in enhancing viable stem cell retention during treatments for cardiac repair. In one study, human mesenchymal stem cells (hMSCs) were encapsulated in alginate hydrogels for use in a rat myocardial infarction model. These encapsulated cells were attached to the heart with a biocompatible PEG hydrogel patch, allowing cell contact with the injured heart. It was shown that the encapsulation of the hMSCs allowed for improved retention of the cells and facilitated desired paracrine effects, such as decreased scarring and increased peri-infarct microvasculature (60). Mayfield et al (61) encapsulated proliferated cardiac stem cells for injection, which showed improved cardiac structure and function over the control group. In the future, the systems developed in these 2 studies could be further tested by using regular rats to monitor their performance under the host immune response. In addition to using this technology for cardiac repair, biocapsules have been used for constructing beating cardiac tissue. Some of the most common bioencapsulation systems used in these cardiac applications are alginate-poly-L-lysine (62) and alginate core-shell microcapsules (7, 8).

Pancreatic and hepatic tissue engineering

Cell encapsulation has the potential to aid in the treatment of type 1 diabetes. Current treatment methods do not effectively treat the disease, instead they only inhibit its progression, showing that new treatment methods would be desirable (63). A direct approach to treat the damaged endocrine tissue is whole pancreas transplantation, which can improve the quality of life for the patient. There are risks associated with the surgery, and the number of available transplant-quality pancreases is low, so it is not an option for most patients with type 1 diabetes (64). A more viable option for treatment is the transplantation of the pancreatic islets. Islets can be isolated, quantified and transplanted into the human body to aid in modulating glucose levels. However, these islets cause immune reactions in a

foreign host. With cell encapsulation, the islets can be immunoisolated to enhance the efficacy of this treatment and eliminate the need for the patient to undergo chronic immunosuppression. This strategy has been proven successful in animal models and has begun to see success in human trials as well. When human islets were extracted and immunoisolated with the alginate-PLO-alginate system for treatment, the patients involved had improved glycemic control after 1 year without reporting any adverse effects. The patients still required exogenous insulin therapy, but the weekly hypoglycemic episodes were eliminated, indicating an improvement of the disease (50). One recent study reported a novel design which combines bioencapsulation and PEGylation for immunocamouflaging the islets of Langerhans (65). With the progress of stem cell research, stem cells could be differentiated to insulin-producing cells (66), which could be used as a new cell source for pancreatic tissue engineering. Interestingly, it has been recently demonstrated that hydrogel microencapsulated insulin-secreting cells can accelerate wound healing in a diabetic mouse model (67).

Liver disease and the subsequent loss of liver function is currently the 12th most frequent cause of death in the United States and the 4th most frequent for middle-aged adults (68). There are several published studies that use bioencapsulation technology for the treatment of acute hepatic failure (AHF) and hepatic injury (69–71). Transplantation of alginate-poly-L-lysine-alginate (APA) microcapsules containing a mixture of rat hepatocytes and human fetal liver stromal cells (hFLSCs), engineered to produce basic fibroblast growth factor (bFGF), in mice increased the survival rate and improved liver function of an acute liver failure induced mouse model. Moreover, significant liver regeneration was observed 2 days after transplantation in the bioencapsulation group (69). Zhang et al (70) reported the encapsulation of hepatocyte-like cells differentiated from human umbilical cord blood cells in Caalginate microbeads and transplantation of the encapsulated cells intraperitoneally into rats with galactosamine-induced AHF. The results showed that the number of surviving rats increased due to the alleviation of AHF, compared with control rats 2 days following transplantation. In addition, transplantation of umbilical cord blood cells encapsulated in APA microcapsules was proven to enhance recovery of CCl4-injured mouse livers (71).

Lung tissue engineering

Recently, bioencapsulation technologies have been applied in controlling the formation of alveolus-like structures in vivo, as shown in a study by Zhang et al (19). In their study, collagen-Matrigel and APA microcapsules were used as an extracellular matrix (ECM) to provide a 3D culture condition to reconstruct the alveolus-like structure (Fig. 5). This 3D culture method was confirmed as providing mice fetal pulmonary cells with a stable growth condition, aiding in the formation of the alveolus-like structures and maintaining an alveolar type II (AE2) differentiated state. AE2 cells are considered the stem cell–like population present in the lung and are significant in the repair and regeneration of lung tissue. After 7 and 14 days of culture, histology and immunohistochemistry of the cultures revealed branching, spherical, hollow structures similar to native mouse lung, as well as revealing alveolus-like structures. Transmission electron microscopy studies verified the presence of sporadic lamellar bodies, which are indicative of AE2 cells maintaining their differentiated

state. It is worth mentioning that this type of engineered ECM, combined with vein endothelial cells, shows great potential in constructing microvascularized 3D tissues.

Bioprinting

Bioencapsulation has also been combined with bioprinting for the advancement of tissue engineering. One example is shown in Figure 6, where chondrocytes were seeded into a bioabsorbable alginate hydrogel matrix before 3D printing. This process localized the cells into a desired geometry, allowing for new ECM production in defined locations and eliminating the major problems usually associated with bioprinting, such as seeding depth limitations and nonuniform seeding (72). Bioprinting of a nanofiber matrix embedded with encapsulated cells could be used to create a smart “cell sheet” with desired pore sizes as a ready-to-use cell source.

Cell and tissue cryopreservation

Successful cryopreservation of cells and tissues can promote their availability as cell-based medicines by establishing banks of living cells for wide distribution to end users whenever needed. While current preservation methods can reduce the cell viability, bioencapsulation provides a novel and alternative route for cell and tissue cryopreservation including vitrification. Zhang et al (13) successfully demonstrated that small (~100 μm) Ca-alginate microcapsules provide a great system for protecting cells from cryoinjury during cryopreservation. Huang et al (73) confirmed that alginate microencapsulation allows large-volume cell vitrification with low concentration of cryoprotectants. Ba-alginate hydrogel, another alginate-based encapsulation system, has also been used for the cryopreservation of neurospheres (12). Moreover, the application of cryopreserved transgenic mesenchymal stem cell–loaded capsules (500–600 μm) in intracerebral hemorrhage treatment has entered clinical trials (32).

Challenges and future directions

Although bioencapsulation technologies show great promise for tissue engineering, there are still several issues that need to be addressed for eventual clinical applications, including limited cell resources, protrusion of encapsulated cells and scaling-up (especially following Good Manufacturing Practice (GMP) guidelines) (2, 23, 74–76). The recent progress of stem cell research prominently expands cell resources for bioencapsulation, addressing the first limitation (77). To overcome the issues pertaining to protrusion of encapsulated cells and scaling-up, current studies are ongoing. Most notably, a novel multilayer immunoisolating encapsulation system is being developed to prevent cell protrusion without compromising cell survival (75), and a 3D microfluidic device containing an air supply and multinozzle outlet is being studied for scaling-up the process (78).

Two future directions for bioencapsulation technologies are the combination with microtechnologies and nanotechnologies and construction of vascularized tissues. An example of a current combination of technologies is the use of nanofibers for reinforcing the hydrogel in the encapsulation process (79, 80). It is well known that vascularization is the major challenge in tissue engineering (81), leading research into bioencapsulation

technologies to focus on this area. Using microcapsules to reinforce the ECM shows the potential for constructing vascularized tissues in which microcapsules could have a space-occupying effect and serve as a seeding cell growth scaffold (19). It is predicted that future advancements of bioencapsulation technologies will focus further on these areas.

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References

1. Gurruchaga H, Saenz del Burgo L, Ciriza J, Orive G, Hernández RM, Pedraz JL. Advances in cell encapsulation technology and its application in drug delivery. Expert Opin Drug Deliv. 2015; 12(8): 1251–1267. [PubMed: 25563077]
2. Murua A, Portero A, Orive G, Hernández RM, de Castro M, Pedraz JL. Cell microencapsulation technology: towards clinical application. J Control Release. 2008; 132(2):76–83. [PubMed: 18789985]
3. Orive G, Hernández RM, Gascón AR, et al. Cell encapsulation: promise and progress. Nat Med. 2003; 9(1):104–107. [PubMed: 12514721]
4. Zhang W, He X. Microencapsulating and banking living cells for cell-based medicine. J Healthc Eng. 2011; 2(4):427–446. [PubMed: 22180835]
5. Orive G, Santos E, Pedraz JL, Hernández RM. Application of cell encapsulation for controlled delivery of biological therapeutics. Adv Drug Deliv Rev. 2014; 67–68:3–14.
6. Zhang W, Zhao S, He X. Proliferation and differentiation of mesenchymal stem cells encapsulated in miniaturized 3D liquid core of alginate-chitosan-alginate (ACA) microcapsules. Archives of Stem Cell Research. 2015; 2(1):1004.
7. Agarwal P, Zhao S, Bielecki P, et al. One-step microfluidic generation of pre-hatching embryo-like core-shell microcapsules for miniaturized 3D culture of pluripotent stem cells. Lab Chip. 2013; 13(23):4525–4533. [PubMed: 24113543]
8. Zhao S, Agarwal P, Rao W, et al. Coaxial electrospray of liquid core-hydrogel shell microcapsules for encapsulation and miniaturized 3D culture of pluripotent stem cells. Integr Biol (Camb). 2014; 6(9):874–884. [PubMed: 25036382]
9. Zhang W, Zhao S, Rao W, et al. A novel core-shell microcapsule for encapsulation and 3d culture of embryonic stem cells. J Mater Chem B Mater Biol Med. 2013; 2013(7):1002–1009. [PubMed: 23505611]
10. Wang X, Wang W, Ma J, Guo X, Yu X, Ma X. Proliferation and differentiation of mouse embryonic stem cells in APA microcapsule: a model for studying the interaction between stem cells and their niche. Biotechnol Prog. 2006; 22(3):791–800. [PubMed: 16739963]
11. Murua A, Orive G, Hernández RM, Pedraz JL. Cryopreservation based on freezing protocols for the long-term storage of microencapsulated myoblasts. Biomaterials. 2009; 30(20):3495–3501. [PubMed: 19327822]
12. Malpique R, Osório LM, Ferreira DS, et al. Alginate encapsulation as a novel strategy for the cryopreservation of neurospheres. Tissue Eng Part C Methods. 2010; 16(5):965–977. [PubMed: 20001270]
13. Zhang W, Yang G, Zhang A, Xu LX, He X. Preferential vitrification of water in small alginate microcapsules significantly augments cell cryopreservation by vitrification. Biomed Microdevices. 2010; 12(1):89–96. [PubMed: 19787454]
14. Wikström J, Elomaa M, Nevala L, et al. Viability of freeze dried microencapsulated human retinal pigment epithelial cells. Eur J Pharm Sci. 2012; 47(2):520–526. [PubMed: 22820032]

15. Gurruchaga H, Ciriza J, Saenz Del Burgo L, et al. Cryopreservation of microencapsulated murine mesenchymal stem cells genetically engineered to secrete erythropoietin. Int J Pharm. 2015; 485(1–2):15–24. [PubMed: 25708005]
16. Harvestine JN, Mikulski BA, Mahuta KM, et al. A novel red-blood-cell-shaped pectin-oligochitosan hydrogel system. Part Part Syst Charact. 2014; 31(9):955–959.
17. Crouse JZ, Mahuta KM, Mikulski BA, et al. Development of a microscale red blood cell-shaped pectin-oligochitosan hydrogel system using an electrospray-vibration method: preparation and characterization. J Appl Biomater Funct Mater. 2015; 13(4):e326–e331. [PubMed: 26616754]
18. Chang, TMS. Artificial cells: biotechnology, nanomedicine, regenerative medicine, blood substitutes, bioencapsulation, cell/stem cell therapy. Vol. 1. Singapore: World Scientific Publishing; 2007.
19. Zhang WJ, Lin QX, Zhang Y, et al. The reconstruction of lung alveolus-like structure in collagen-matrigel/microcapsules scaffolds in vitro. J Cell Mol Med. 2011; 15(9):1878–1886. [PubMed: 21029367]
20. Gasperini L, Mano JF, Reis RL. Natural polymers for the microencapsulation of cells. J R Soc Interface. 2014; 11(100):20140817. [PubMed: 25232055]
21. Zhang WJ, Li BG, Zhang C, Xie XH, Tang TT. Biocompatibility and membrane strength of C3H10T1/2 cell-loaded alginate-based microcapsules. Cytotherapy. 2008; 10(1):90–97. [PubMed: 18202978]
22. Orive G, Santos E, Poncelet D, et al. Cell encapsulation: technical and clinical advances. Trends Pharmacol Sci. 2015; 36(8):537–546. [PubMed: 26067102]
23. Zhang, W. Encapsulation of transgenic cells for gene therapy, Gene Therapy: principles and challenges. Hashad, D., editor. InTech; InTech: Rijeka, Croatia; 2015.
24. Santos E, Zarate J, Orive G, Hernández RM, Pedraz JL. Biomaterials in cell microencapsulation. Adv Exp Med Biol. 2010; 670:5–21. [PubMed: 20384214]
25. Olabisi RM. Cell microencapsulation with synthetic polymers. J Biomed Mater Res A. 2015; 103(2):846–859. [PubMed: 24771675]
26. Bidarra SJ, Barrias CC, Granja PL. Injectable alginate hydrogels for cell delivery in tissue engineering. Acta Biomater. 2014; 10(4):1646–1662. [PubMed: 24334143]
27. Gattás-Asfura K, Valdes M, Celik E, Stabler C. Covalent layer-by-layer assembly of hyperbranched polymers on alginate microcapsules to impart stability and permselectivity. J Mater Chem B Mater Biol Med. 2014; 2(46):8208–8219. [PubMed: 25478165]
28. Park HS, Kim JW, Lee SH, et al. Antifibrotic effect of rapamycin containing polyethylene glycol-coated alginate microcapsule in islet xenotransplantation. J Tissue Eng Regen Med. 2015 Jun 5. [Epub ahead of print].
29. Zanotti L, Sarukhan A, Dander E, et al. Encapsulated mesenchymal stem cells for in vivo immunomodulation. Leukemia. 2013; 27(2):500–503. [PubMed: 22878604]
30. Köllmer M, Appel AA, Somo SI, Brey EM. Long-term function of alginate-encapsulated islets. Tissue Eng Part B@@Rev. 2015; 22(1):34–46.
31. Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation. 2007; 14(2): 157–161. [PubMed: 17381690]
32. CellMed AG. [Accessed May 13, 2016] GLP-1 CellBeads® for the Treatment of Stroke Patients With Space-Occupying Intracerebral Hemorrhage. NCT01298830. http://www.clinicaltrials.gov
33. Living Cell Technologies. [Accessed May 13, 2016] Open-label Investigation of the Safety and Clinical Effects of NTCELL in Patients With Parkinson's Disease. Disease (NCT01734733) and Investigation of the Safety and Efficacy of NTCELL® [Immunoprotected (Alginate-Encapsulated) Porcine Choroid Plexus Cells for Xenotransplantation] in Patients With Parkinson's Disease (NCT02683629). http://www.clinicaltrials.gov
34. Olabisi RM, Lazard ZW, Franco CL, et al. Hydrogel microsphere encapsulation of a cell-based gene therapy system increases cell survival of injected cells, transgene expression, and bone volume in a model of heterotopic ossification. Tissue Eng Part A. 2010; 16(12):3727–3736. [PubMed: 20673027]

35. Mumaw J, Jordan ET, Sonnet C, et al. Rapid heterotrophic ossification with cryopreserved poly(ethylene glycol-) microencapsulated BMP2-expressing MSCs. Int J Biomater. 2012; 2012:861794. [PubMed: 22500171]
36. de Vos P, Lazarjani HA, Poncelet D, Faas MM. Polymers in cell encapsulation from an enveloped cell perspective. Adv Drug Deliv Rev. 2014; 67–68:15–34.
37. Ren X, Feng Y, Guo J, et al. Surface modification and endothelialization of biomaterials as potential scaffolds for vascular tissue engineering applications. Chem Soc Rev. 2015; 44(15): 5680–5742. [PubMed: 26023741]
38. Pramanik S, Ataollahi F, Pingguan-Murphy B, Oshkour AA, Osman NA. In vitro study of surface modified poly(ethylene glycol)-impregnated sintered bovine bone scaffolds on human fibroblast cells. Sci Rep. 2015; 5:9806. [PubMed: 25950377]
39. M Jonker A, A Bode S, H Kusters A, van Hest JC, Löwik DW. Soft PEG-Hydrogels with independently tunable stiffness and rgds-content for cell adhesion studies. Macromol Biosci. 2015; 15(10):1338–1347. [PubMed: 26097013]
40. McKinnon DD, Kloxinb AM, Anseth KS. Synthetic hydrogel platform for three-dimensional culture of embryonic stem cell-derived motor neurons. Biomater Sci. 2013; 1(5):460–469.
41. Lathuilière A, Cosson S, Lutolf MP, Schneider BL, Aebischer P. A high-capacity cell macroencapsulation system supporting the long-term survival of genetically engineered allogeneic cells. Biomaterials. 2014; 35(2):779–791. [PubMed: 24103654]
42. Teramura Y, Minh LN, Kawamoto T, Iwata H. Microencapsulation of islets with living cells using polyDNA-PEG-lipid conjugate. Bioconjug Chem. 2010; 21(4):792–796. [PubMed: 20210336]
43. Sarker B, Rompf J, Silva R, et al. Alginate-based hydrogels with improved adhesive properties for cell encapsulation. Int J Biol Macromol. 2015; 78:72–78. [PubMed: 25847839]
44. Dumbleton J, Agarwal P, Huang H, et al. The effect of RGD peptide on 2D and miniaturized 3D culture of HEPM cells, MSCs, and ADSCs with alginate hydrogel. Cell Mol Bioeng. 2016 [Epub ahead of print].
45. Teramura Y, Ekdahl KN, Barbu A. A hybrid of cells and pancreatic islets toward a new bioartificial pancreas. Regenerative Therapy. 2016; 3:68–74.
46. Takemoto N, Teramura Y, Iwata H. Immobilization of Sertoli cells on islets of Langerhans. Biomaterials Science. 2013; 1(3):315–321.
47. Kang A, Park J, Ju J, Jeong GS, Lee SH. Cell encapsulation via microtechnologies. Biomaterials. 2014; 35(9):2651–2663. [PubMed: 24439405]
48. Zhang W, He X. Encapsulation of living cells in small (approximately 100 microm) alginate microcapsules by electrostatic spraying: a parametric study. J Biomech Eng. 2009; 131(7):074515. [PubMed: 19640151]
49. Mazzitelli S, Capretto L, Quinci F, Piva R, Nastruzzi C. Preparation of cell-encapsulation devices in confined microenvironment. Adv Drug Deliv Rev. 2013; 65(11–12):1533–1555. [PubMed: 23933618]
50. Steele JA, Hallé JP, Poncelet D, Neufeld RJ. Therapeutic cell encapsulation techniques and applications in diabetes. Adv Drug Deliv Rev. 2014; 67–68:74–83.
51. Koch S, Schwinger C, Kressler J, Heinzen Ch, Rainov NG. Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. J Microencapsul. 2003; 20(3):303–316. [PubMed: 12881112]
52. Strand BL, Gåserød O, Kulseng B, Espevik T, Skjåk-Baek G. Alginate-polylysine-alginate microcapsules: effect of size reduction on capsule properties. J Microencapsul. 2002; 19(5):615–630. [PubMed: 12433304]
53. Schwinger C, Koch S, Jahnz U, Wittlich P, Rainov NG, Kressler J. High throughput encapsulation of murine fibroblasts in alginate using the JetCutter technology. J Microencapsul. 2002; 19(3): 273–280. [PubMed: 12022493]
54. Kim C, Park J, Kang JY. A microfluidic manifold with a single pump system to generate highly mono-disperse alginate beads for cell encapsulation. Biomicrofluidics. 2014; 8(6):066504. [PubMed: 25587376]
55. Kim C, Lee KS, Kim YE, et al. Rapid exchange of oil-phase in microencapsulation chip to enhance cell viability. Lab Chip. 2009; 9(9):1294–1297. [PubMed: 19370252]

56. BÜCHI Labortechnik AG. Flawi, Switzerland: BÜCHI Labortechnik AG; Encapsulator B-390: the valued bead and capsule producer [company website page]. http://www.buchi.com/en/products/spray-drying-and-encapsulation/encapsulator-b-390 [Accessed May 13, 2016]
57. Cellena. Sevilla, Spain: Ingeniatrics Technologies; Portable microencapsulation equipment Cellena® [company website page]. http://www.cellena.net/en/portable_microencapsulation_equipment_cellena.html [Accessed May 13, 2016]
58. Fang C, Wang D-A. A biodegradable PEG-based micro-cavitary hydrogel as scaffold for cartilage tissue engineering. Eur Polym J. 2015; 72:651–660.
59. Tang M, Chen W, Weir MD, Thein-Han W, Xu HH. Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering. Acta Biomater. 2012; 8(9):3436–3445. [PubMed: 22633970]
60. Levit RD, Landázuri N, Phelps EA, et al. Cellular encapsulation enhances cardiac repair. J Am Heart Assoc. 2013; 2(5):e000367. [PubMed: 24113327]
61. Mayfield AE, Tilokee EL, Latham N, et al. The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. Biomaterials. 2014; 35(1):133–142. [PubMed: 24099706]
62. Jing D, Parikh A, Tzanakakis ES. Cardiac cell generation from encapsulated embryonic stem cells in static and scalable culture systems. Cell Transplant. 2010; 19(11):1397–1412. [PubMed: 20587137]
63. Kobayashi N. Bioartificial pancreas for the treatment of diabetes. Cell Transplant. 2008; 17(1–2): 11–17. [PubMed: 18468230]
64. Stegall MD, Dean PG, Sung R, et al. The rationale for the new deceased donor pancreas allocation schema. Transplantation. 2007; 83(9):1156–1161. [PubMed: 17496529]
65. Nabavimanesh MM, Hashemi-Najafabadi S, Vasheghani-Farahani E. Islets immunoisolation using encapsulation and PEGylation, simultaneously, as a novel design. J Biosci Bioeng. 2015; 119(4): 486–491. [PubMed: 25454695]
66. Kaitsuka T, Noguchi H, Shiraki N, et al. Generation of functional insulin-producing cells from mouse embryonic stem cells through 804G cell-derived extracellular matrix and protein transduction of transcription factors. Stem Cells Transl Med. 2014; 3(1):114–127. [PubMed: 24292793]
67. Aijaz A, Faulknor R, Berthiaume F, Olabisi RM. Hydrogel microencapsulated insulin-secreting cells increase keratinocyte migration, epidermal thickness, collagen fiber density, and wound closure in a diabetic mouse model of wound healing. Tissue Eng Part A. 2015; 21(21–22):2723–2732. [PubMed: 26239745]
68. Bhatia SN, Underhill GH, Zaret KS, Fox IJ. Cell and tissue engineering for liver disease. Sci Transl Med. 2014; 6(245):245sr2. [PubMed: 25031271]
69. Teng Y, Wang Y, Li S, et al. Treatment of acute hepatic failure in mice by transplantation of mixed microencapsulation of rat hepatocytes and transgenic human fetal liver stromal cells. Tissue Eng Part C Methods. 2010; 16(5):1125–1134. [PubMed: 20121581]
70. Zhang FT, Wan HJ, Li MH, et al. Transplantation of microencapsulated umbilical-cord-blood-derived hepatic-like cells for treatment of hepatic failure. World J Gastroenterol. 2011; 17(7):938–945. [PubMed: 21412504]
71. Li S, Sun Z, Lv G, et al. Microencapsulated UCB cells repair hepatic injure by intraperitoneal transplantation. Cytotherapy. 2009; 11(8):1032–1040. [PubMed: 19929467]
72. Mannoor MS, Jiang Z, James T, et al. 3D printed bionic ears. Nano Lett. 2013; 13(6):2634–2639. [PubMed: 23635097]
73. Huang H, Choi JK, Rao W, et al. Alginate hydrogel microencapsulation inhibits devitrification and enables large-volume low-CPA cell vitrification. Adv Funct Mater. 2015; 25(44):6939–6850. [PubMed: 26640426]
74. Santos E, Pedraz JL, Hernández RM, Orive G. Therapeutic cell encapsulation: ten steps towards clinical translation. J Control Release. 2013; 170(1):1–14. [PubMed: 23643824]
75. Bhujbal SV, de Haan B, Niclou SP, de Vos P. A novel multilayer immunoisolating encapsulation system overcoming protrusion of cells. Sci Rep. 2014; 4:6856. [PubMed: 25358640]

76. Villani S, Marazzi M, Bucco M, et al. Statistical approach in alginate membrane formulation for cell encapsulation in a GMP-based cell factory. Acta Biomater. 2008; 4(4):943–949. [PubMed: 18296132]

77. Komatsu M, Konagaya S, Egawa EY, Iwata H. Maturation of human iPS cell-derived dopamine neuron precursors in alginate-Ca(2+) hydrogel. Biochim Biophys Acta. 2015; 1850(9):1669–1675. [PubMed: 25952075]

78. Tendulkar S, Mirmalek-Sani SH, Childers C, Saul J, Opara EC, Ramasubramanian MK. A three-dimensional microfluidic approach to scaling up microencapsulation of cells. Biomed Microdevices. 2012; 14(3):461–469. [PubMed: 22245953]

79. An D, Ji Y, Chiu A, et al. Developing robust, hydrogel-based, nanofiber-enabled encapsulation devices (NEEDs) for cell therapies. Biomaterials. 2015; 37:40–48. [PubMed: 25453936]

80. Huang X, Wang J, Xie H, et al. Microcapsules embedded with three-dimensional fibrous scaffolds for cell culture and tissue engineering. Tissue Eng Part C Methods. 2010; 16(5):1023–1032. [PubMed: 20050807]

81. Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. Adv Drug Deliv Rev. 2011; 63(4–5):300–311. [PubMed: 21396416]

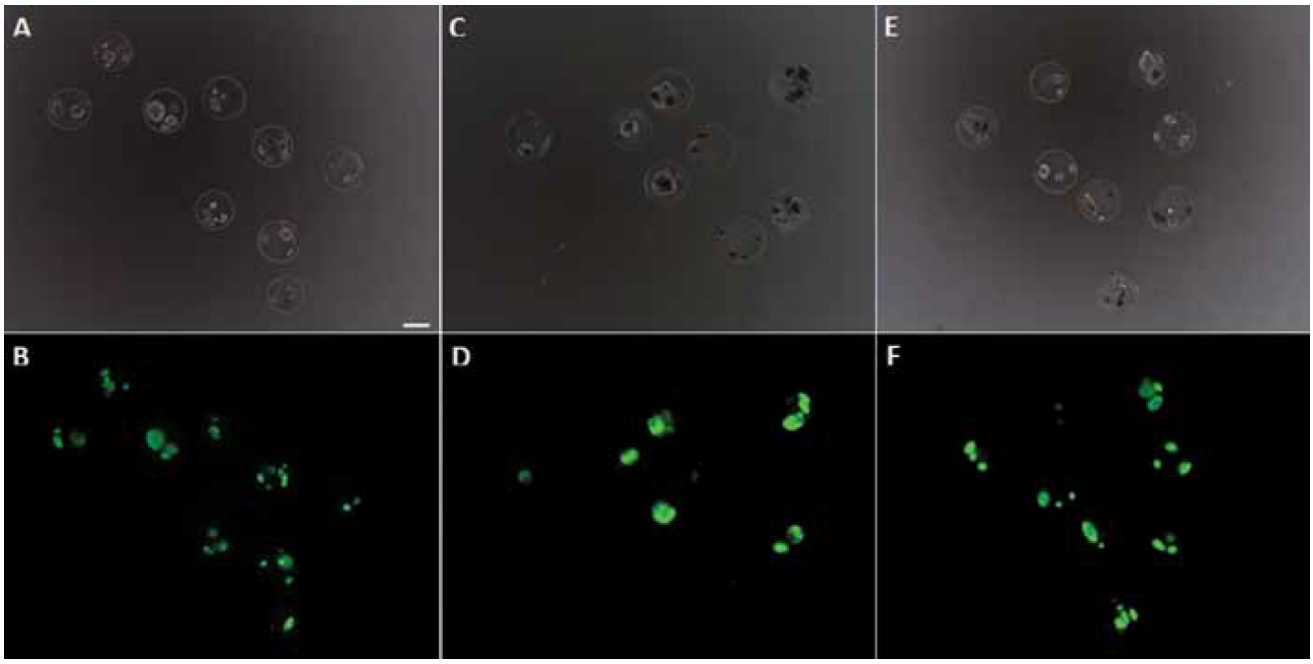

Fig. 1.

Phase contrast (**A, C, E**) and corresponding fluorescence (**B, D, F**) images of encapsulated mesenchymal stem cells (C3H10T1/2 cells) cultured under different conditions: cells cultured in Dulbecco's modified Eagle's medium (DMEM) for 7 weeks (**A, B**); cells cultured in DMEM for 4 weeks and then in adipogenic differentiation medium for 3 weeks (**C, D**); and cells cultured in DMEM for 4 weeks and then in osteogenic differentiation medium for 3 weeks (**E, F**). Reproduced from reference (6). In the fluorescence images, live and dead cells were stained green and red, respectively. Scale bar: 100 μm.

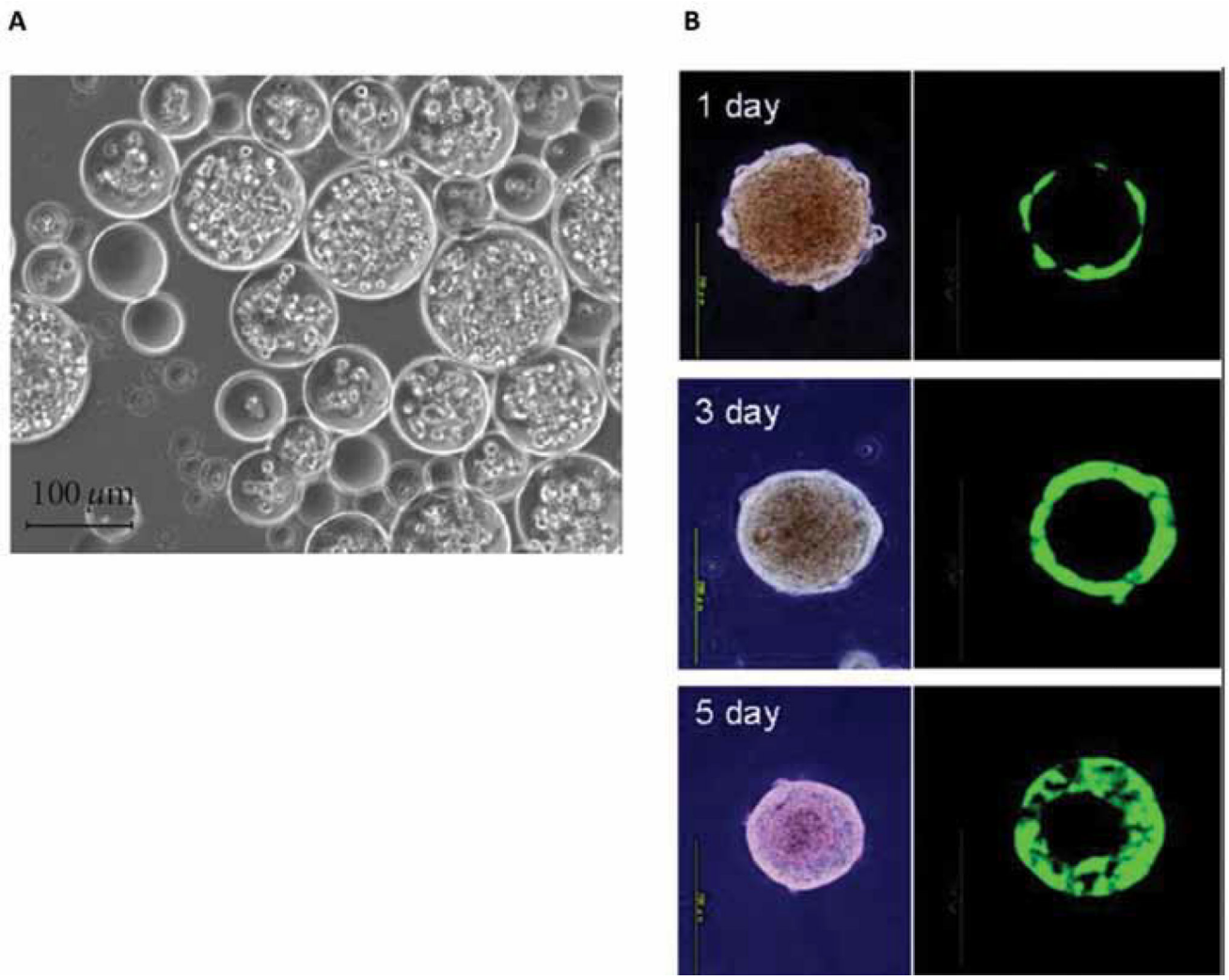

Fig. 2.

(**A**) Phase contrast image of poly(ethylene glycol)-microencapsulated mesenchymal stem cells for bone tissue engineering. Reproduced from reference (35). (**B**) Green fluorescent protein (GFP)–HEK cell–encapsulated islets were cultured for 1, 3 and 5 days. Encapsulated Islets were observed with a phase contrast microscope (left panels) and a confocal laser scanning microscope (right panels; GFP). Scale bars: 200 μm. Adapted with permission from Yuji Teramura, Luan Nguyen Minh, Takuo Kawamoto and Hiroo Iwata. Microencapsulation of islets with living cells using polyDNA-PEG-lipid conjugate. *Bioconjugate Chemistry*. 2010;21(4):792–796. doi:10.1021/bc900494x. Copyright 2010 by the American Chemical Society (42).

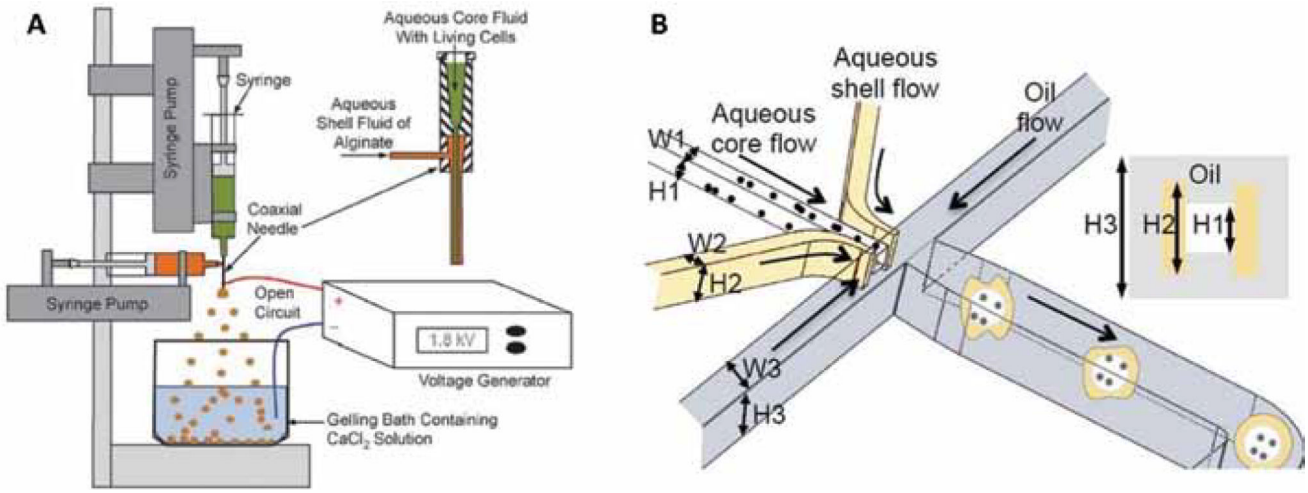

Fig. 3.

Novel designs for producing core-shell microcapsules. (**A**) An electrospray device–based system: the core fluid (sodium carboxymethyl cellulose–containing cells) and shell fluid (alginate) are separately pumped through the concentric needle. Under the effect of an electric field, the concentric drops that form at the tip of the needle are broken up into microdrops and sprayed into the gelling bath, ultimately producing core-shell microcapsules (8) -Reproduced by permission of The Royal Society of Chemistry. (**B**) A microfluidics device–based system: at the focusing junction, both the core (sodium carboxymethyl cellulose–containing cells) and shell (alginate) flows were sheared by the crossing oil flow (mineral oil containing calcium chloride) into droplets. Then, calcium cations diffused into the droplets and gelled alginate, thus forming the microcapsule shell (7)-Reproduced by permission of The Royal Society of Chemistry. H: height (or depth); W: width.

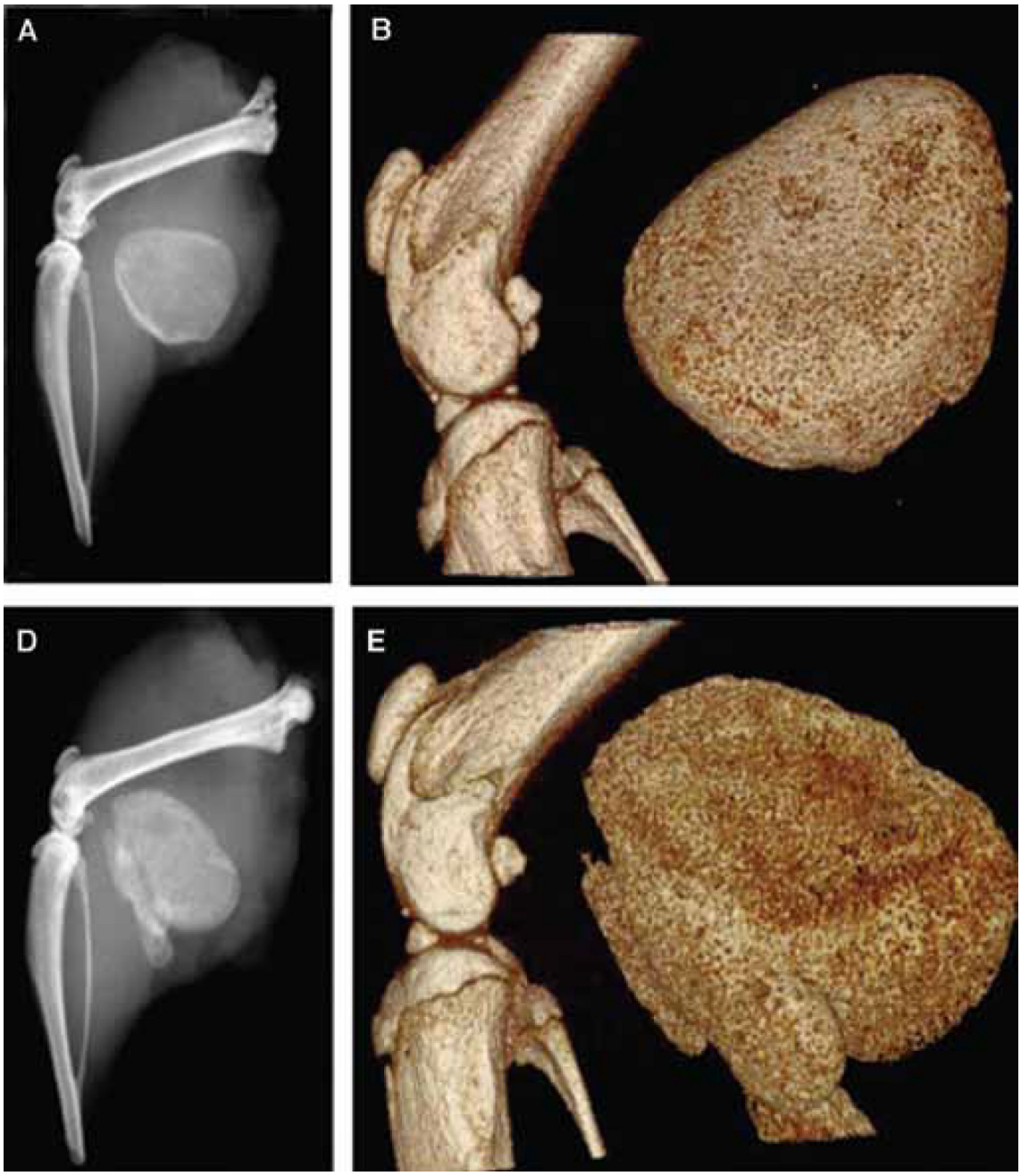

Fig. 4.

Bone morphogenetic protein 2 (BMP2)–transduced microencapsulated mesenchymal stem cell (MSC) bone formation in a mouse model for heterotopic ossification confirmed by both X-ray (**A, D**) and MicroCT for (**B, E**). Top panel: freshly prepared BMP2-microencapsulated MSCs; Bottom panel: cryopreserved BMP2-microencapsulated MSCs. Reproduced from reference (35).

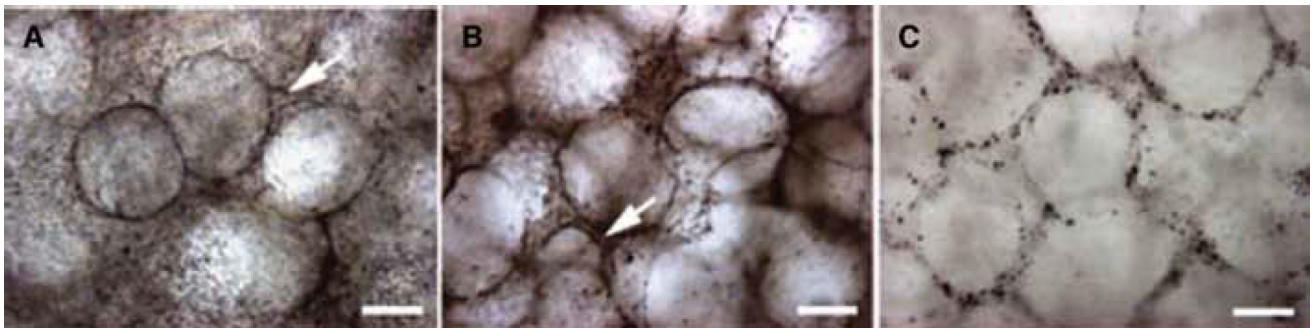

Fig. 5.

Reconstructed alveolus-like structures in vitro were observed under phase contrast microscope. The structures were observed after 7 (**A**), 14 (**B**) and 21 (**C**) days. Reproduced from reference (19) with permission from Wiley & Sons Ltd. Cells gathered to grow (white arrow) could be observed in some parts of the alveolus-like structures (**B**). Scale bar: 200 μm.

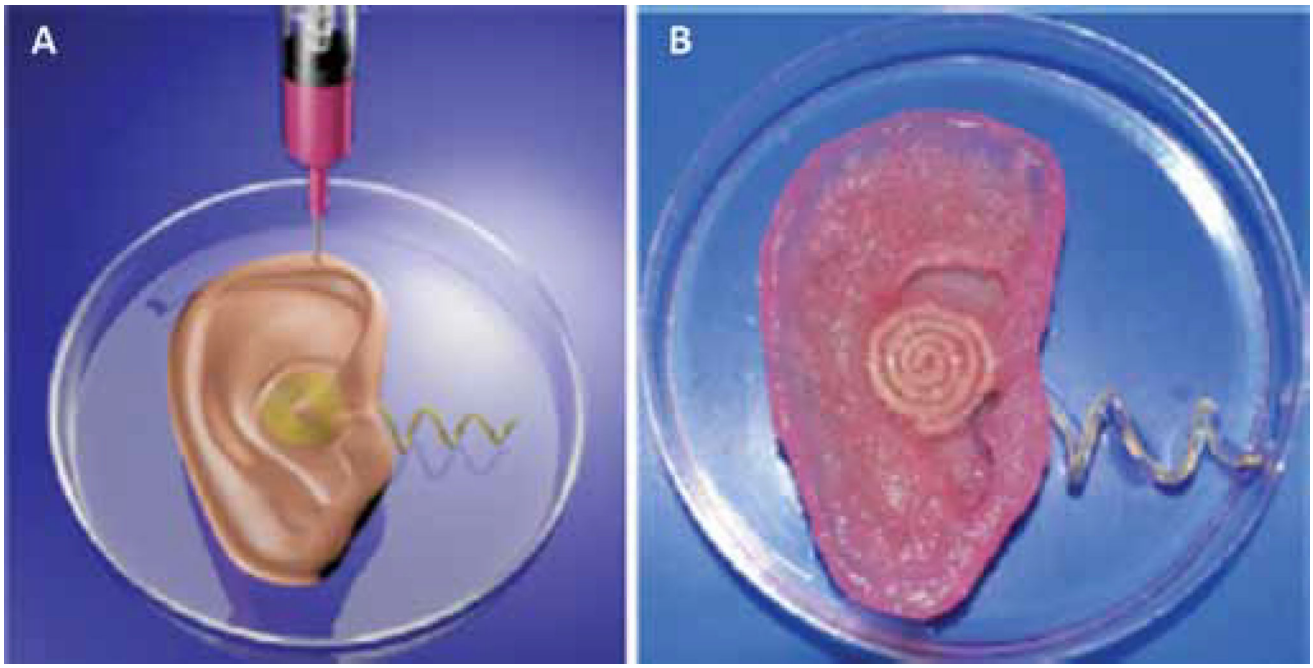

Fig. 6.

Growth and viability of the bionic ear. (**A**) Image of the 3D printed bionic ear immediately after printing. (**B**) Image of the 3D printed bionic ear during in vitro culture. Adapted with permission from Manu S. Mannoor, Ziwen Jiang, Teena James, Yong Lin Kong, Karen A. Malatesta, Winston O. Soboyejo, Naveen Verma, David H. Gracias and Michael C. McAlpine. 3D printed bionic ears. *Nano Letters*. 2013;13(6):2634–2639. Copyright 2015 by the American Chemical Society (72).