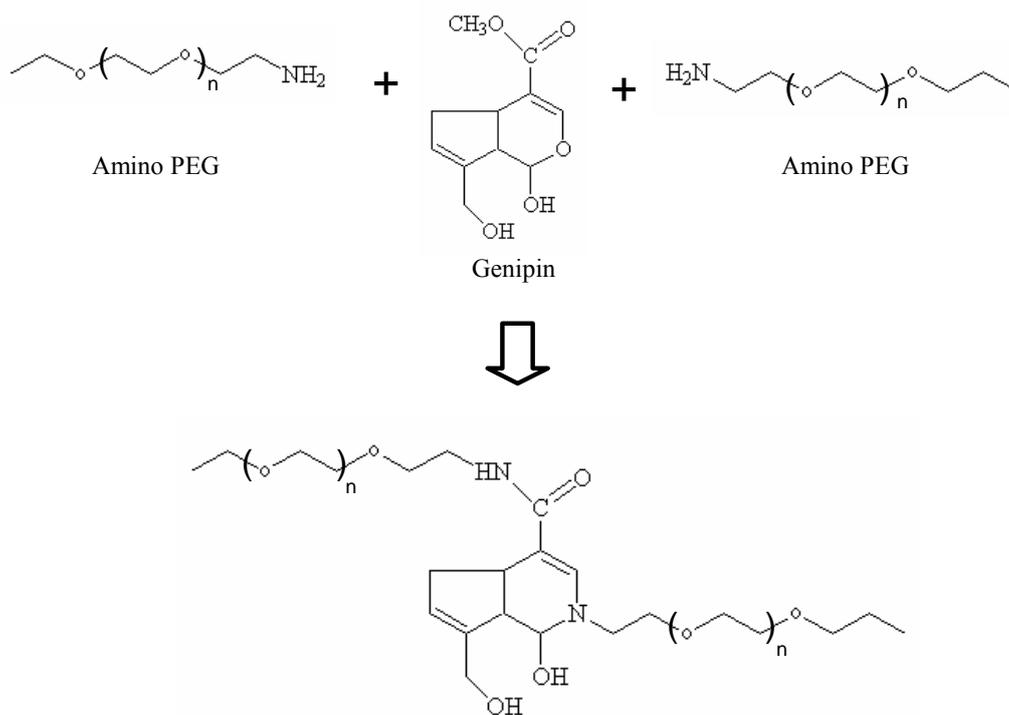


longer gelation time is required for hydrogel formation due to its relatively lower reactivity of the methacryl group. To further accelerate the gelation of the hydrogel for cell delivery procedure, an alternative system based upon the conjugate addition crosslinking between thiol-modified HA and PEGDA was developed, which satisfies most key requirements for injectable *in vivo* tissue engineering applications [115–116].

Figure 3. The reaction scheme of amino PEG-genipin hydrogel.



3.2.3. Genipin crosslinked hydrogels

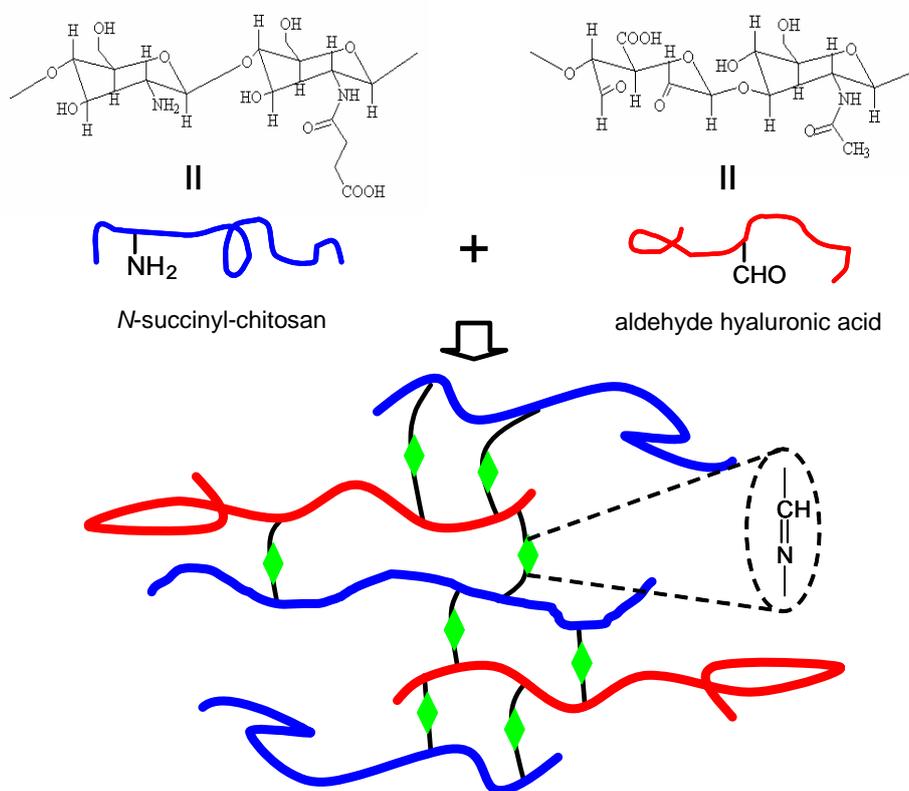
Genipin is a natural product extracted from the gardenia fruit, which overcomes the toxicity inherent in most commonly used synthetic cross-linkers [118–119]. Recent studies identified that genipin can be utilized to crosslink functional amine groups present in natural tissues and biomaterials with very minimal cytotoxic effects, compared to studies performed with glutaraldehyde, a commonly used crosslinker [119–123], resulting in materials with a deep blue color. The utilization of genipin (0.5–3.5 wt %) to crosslink natural biocompatible polymers, such as chitosan and gelatin, to form biodegradable hydrogels has the potential to produce novel scaffolds for various tissue engineering applications. Our laboratory has recently examined the synthesis of novel amino-terminated multi-arm PEG based hydrogels utilizing genipin as a crosslinking agent [124–127]. We examined 2 PEG structures: 4-arm PEG, a molecule with 4 PEG chains attached at a central point, and 8-arm PEG, a molecule with 8 PEG chains attached to a central molecule (Figure 3). The gelation time, swelling, water uptake and weight loss were dependent on the structure of the PEG hydrogel. Due to the molecular architecture, the 8-arm PEG hydrogel showed a much slower gelation reaction, more compact structure and lower water uptake than those of the 4-arm PEG hydrogel, as well as a better stability *in vitro* with a 35.2 mM genipin. Furthermore, human adipose derived stem cell study results indicated that both the 4-arm and 8-arm PEG hydrogels are able to support cell adhesion. This study

represents the potential opportunity to use genipin cross-linked, multi-arm PEG-genipin hydrogels, especially the 4-arm PEG-genipin, as an injectable scaffold in a variety of tissue engineering applications.

3.2.4. Schiff-base crosslinked hydrogels

More recently, we have developed a new injectable, *in situ* forming biocompatible and biodegradable hydrogel as cell carriers for tissue engineering applications [128]. The polysaccharide hydrogel derived from water-soluble chitosan and oxidized hyaluronic acid gel upon mixing, without employing any extraneous chemical crosslinking agents. The gelation is attributed to the Schiff-base reaction between amino groups of *N*-Succinyl-chitosan and aldehyde groups of oxidized hyaluronic acid (Figure 4). *N*-Succinyl-chitosan, a water soluble chitosan derivative, was synthesized *via* introduction of succinyl groups at the *N*-position of the glucosamine units of chitosan. Hyaluronic acid can be oxidized, and the carbon-carbon bonds of the cis-diol groups in molecular chain are cleaved and generate reactive aldehyde functions, which can develop chemical crosslinking action with amino functions *via* Schiff-base linkage. This polysaccharide hydrogel creates a biomimetic microenvironment with improved biocompatibility and biodegradation for tissue regeneration. Several other polysaccharides such as dextran, gum arabic and chondroitin sulfate can be partially oxidized and employed for Schiff-base linkage [129–134].

Figure 4. The scheme of *N*-succinyl-chitosan and aldehyde hyaluronic acid composite hydrogel *via* Schiff's base cross-linking reaction.



4. Applications of Injectable Hydrogels

4.1. Clinical Applications

The need for injectable, biodegradable hydrogels in biomedical applications is immense. One example is the utility of hydrogels in cartilage regeneration. The physical properties of the hydrogel can be designed to easily match those of articular cartilage in addition to matching mechanical properties of the scaffold with the native tissue. Further applications for hydrogels include soft tissue regeneration after tumor removal or trauma. A number of researchers have studied the combination of injectable hydrogels and biodegradable microspheres for controlled drug delivery in tissue engineering, including our own laboratory [135–139]. The following sections describe the pre-clinical and clinical studies of hydrogels for these applications.

4.2. Cartilage Repair

The need for tissue-engineered cartilage is immense and of great clinical significance. Traumatic and degenerative lesions of articular cartilage are leading causes of disability [140]. It is estimated that over 40 million Americans currently suffer from osteoarthritis [141]. Tissue engineering methods, including the use of injectable hydrogels, to improve cartilage repair and regeneration will therefore have high clinical impact. The advantage of injectable therapies for cartilage repair is that the implant is not only maintained within the defect, but also allows immediate weight-bearing due to the stiffness and strength that is achieved almost instantly. Additionally, a general advantage of injectable therapies is the utilization of minimally invasive surgery as compared to open surgery. As such, there have been numerous studies involving the use of injectable hydrogels for cartilage repair.

4.3. Soft Tissue Regeneration

Soft tissue reconstruction is a significant challenge in reconstructive surgery. There are several reasons for a lack of soft tissue, e.g., adipose tissue, such as congenital (e.g., in Parry-Romberg syndrome [142] or Poland syndrome [143], both of which can result in lipoatrophy), traumatic, or oncologic surgery. Due to a lack of better alternatives, transplantation of autologous adipose tissue has been used for soft tissue reconstruction for the past century. However, the clinical outcome of adipose tissue transplantation is unpredictable as there is variable graft resorption due to a lack of vascularization [144]. A desirable strategy to repair soft tissue is to induce adipogenesis *in situ*. One method to accomplish this is to utilize cells that can differentiate to form adipose tissue, and seed those cells into a scaffold, resulting in adipose tissue formation. Another strategy is to utilize injectable systems. As such, many injectable hydrogels based on both synthetic and natural biomaterials have been examined. For example, Hemmerich *et al.* reported the reconstruction of small defects using injectable hyaluronic acid-based gel which were mixed with undifferentiated adipose-derived stem cells (ASCs). Adequate adipose tissue formation was observed using ASCs and hyaluronic acid as the scaffold [145]. Hyaluronic acid, therefore, is applicable for generating adipose tissue in gels, displaying adipogenic as well as angiogenic properties [146].

Other injectable scaffold matrices include biodegradable, polymeric microspheres. For example, Yuksel *et al.* reported the release of insulin-like growth factor-1 (IGF-1) as well as insulin from PLGA microspheres enhanced *de novo* adipose tissue formation [147]. Their study demonstrated the potential of long-term local IGF-1 and insulin delivery to induce adipogenic differentiation to mature lipid-containing adipocytes from non-adipocyte cell pools (e.g., ASCs) that were administered directly to the deep muscular fascia of the rat abdominal wall.

In addition to PLGA microspheres, the use of extracellular matrix (ECM) particles for injectable systems for adipose tissue engineering has been studied [139,148]. We have previously reported the assessment of ASC attachment, proliferation, and differentiation on gelatinous microparticles, termed CultiSphers [139]. These results demonstrated the potential of using biodegradable particles as cell carriers for soft tissue repair.

5. Conclusions

Injectable scaffolds are promising substrates for tissue engineering with the advantage that drugs and cells can be readily integrated into the gelling matrix. Many efforts have been developed to improve injectable hydrogels and thus, support the development of more natural and functional tissues. The success of injectable tissue constructs is highly dependent on the design of the hydrogel scaffolds including physical, chemical and biological properties. An ideal injectable hydrogel would potentially mimic many roles of ECM found in tissues, resulting in the coexistence of both physical and chemical gels. Current biomaterials are unable to meet all the design parameters simultaneously (e.g., degradation, biocompatibility or mechanical properties). Furthermore, injectable hydrogel development will likely have a significant impact on the advancement of tissue engineering. An objective in future work is to design bioactive materials that would be readily injectable at or below room temperature, would form gels with relatively appropriate biodegradable properties under physiological conditions, and would support cell induction. Novel crosslinking methods should be developed, both to enhance the material biocompatibility as well as control the mechanical properties. In addition, cell induction ligands such as growth factors and genes can be incorporated into the injectable scaffolds such that specific signals could be delivered in an appropriate spatial and temporal manner.

References and Notes

1. Lee, K.Y.; Mooney, D.J. Hydrogels for tissue engineering. *Chem. Rev.* **2001**, *101*, 1869–1879.
2. Drury, J.L.; Mooney, D.J. Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials* **2003**, *24*, 4337–4351.
3. Tememoff, J.S.; Mikos, A.G. Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials* **2000**, *21*, 2405–2412.
4. Hou, Q.P.; De Bank, P.A.; Shakesheff, K.M. Injectable scaffolds for tissue regeneration. *J. Mater. Chem.* **2004**, *14*, 1915–1923.
5. Drury, J.L.; Mooney, D.J. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* **2003**, *24*, 4337–4351.

6. Nuttelman, C.R.; Rice, M.A.; Rydholm, A.E.; Salinas, C.N.; Shah, D.N.; Anseth, K.S. Macromolecular monomers for the synthesis of hydrogel niches and their application in cell encapsulation and tissue engineering. *Prog. Polym. Sci.* **2008**, *33*, 167–179.
7. Brandl, F.; Sommer, F.; Goepferich, A. Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior. *Biomaterials* **2007**, *28*, 134–146.
8. Rehfeldt, R.; Engler, A.J.; Eckhardt, A.; Ahmed, F.; Discher, D.E. Cell responses to the mechanochemical microenvironment--implications for regenerative medicine and drug delivery. *Adv. Drug Deliv. Rev.* **2007**, *59*, 1329–1339.
9. Nicodemus, G.D.; Bryant, S.J. Cell encapsulation in biodegradable hydrogels for tissue Engineering applications. *Tissue Eng.* **2008**, *14*, 149–165.
10. Yu, L.; Ding, J. Injectable hydrogels as unique biomedical materials. *Chem. Soc. Rev.* **2008**, *37*, 1473–1481.
11. Tan, H.; Gong, Y.; Lao, L.; Mao, Z.; Gao, C. Gelatin/chitosan/hyaluronan ternary complex scaffold containing basic fibroblast growth factor for cartilage tissue engineering. *J. Mater. Sci.: Mater. Med.* **2007**, *18*, 1961–1968.
12. Awad, H.A.; Wickham, M.Q.; Leddy, H.A.; Gimble, J.M.; Guilak, F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials* **2004**, *25*, 3211–3222.
13. Tan, H.; Wan, L.; Wu, J.; Gao, C. Microscale control over collagen gradient on poly(L-lactide) membrane surface for manipulating chondrocyte distribution. *Colloids Surf. B: Biointerfaces* **2008**, *67*, 210–215.
14. Lee, C.H.; Singla, A.; Lee, Y. Biomedical applications of collagen. *Int. J. Pharm.* **2001**, *221*, 1–22.
15. Furthmayr, H.; Timol, R. Immunochemistry of collagens and procollagens. *Int. Rev. Connect. Tiss. Res.* **1976**, *7*, 61–99.
16. Yannas, I.V.; Burke, J.F. Design of an artificial skin I. Basic design principles. *J. Biomed. Mater. Res.* **1980**, *14*, 65–81.
17. Stefan, M.M.; Shortkroff, S.; Schneider, T.O.; Breinan, H.A.; Yannas, I.V.; Spector, M. Meniscus cells seeded in type I and type II collagen-GAG matrices *in vitro*. *Biomaterials* **1999**, *20*, 701–709.
18. Tan, H.; Huang, D.; Lao, L.; Gao, C. RGD modified PLGA/gelatin microspheres as microcarriers for chondrocyte delivery. *J. Biomed. Mater. Res.* **2009**, *91B*, 228–238.
19. Huang, Y.; Onyeri, S.; Siewe, M.; Moshfeghian, A.; Madihally, S.V. *In vitro* characterization of chitosan-gelatin scaffolds for tissue engineering. *Biomaterials* **2005**, *26*, 7616–7627.
20. Tan, H.; Lao, L.; Wu, J.; Gong, Y.; Gao, C. Biomimetic modification of chitosan with covalently grafted lactose and blended heparin for improvement of *in vitro* cellular interaction. *Polym. Adv. Technol.* **2008**, *19*, 15–23.
21. Hsieh, W.C.; Chang, C.P.; Lin, S.M. Morphology and characterization of 3D micro-porous structured chitosan scaffolds for tissue engineering. *Colloids Surf. B: Biointerfaces* **2007**, *57*, 250–255.

22. Yuan, Y.; Chesnutt, B.M.; Utturkar, G.; Haggard, W.O.; Yang, Y.; Ong, J.L.; Bumgardner, J.D. The effect of cross-linking of chitosan microspheres with genipin on protein release. *Carbohydr. Polym.* **2007**, *68*, 561–567.
23. Berger, J.; Reist, M.; Mayer, J.M.; Felt, O.; Peppas, N.A.; Gurny, R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Europ. J. Pharm. Biopharm.* **2004**, *57*, 19–34.
24. Mao, J.; Zhao, L.; Yao, K.; Shang, Q.; Yang, G.; Cao, Y. Study of novel chitosan-gelatin artificial skin *in vitro*. *J. Biomed. Mater. Res.* **2003**, *64A*, 301–308.
25. Ma, J.; Wang, H.; He, B.; Chen, J. A preliminary *in vitro* study on the fabrication and tissue engineering applications of a novel chitosan bilayer material as a scaffold of human neonatal dermal fibroblasts. *Biomaterials* **2001**, *22*, 331–337.
26. Chatelet, C.; Damour, O.; Domard, A. Influence of the degree of acetylation on some biological properties of chitosan films. *Biomaterials* **2001**, *22*, 261–268.
27. Goa, K.L.; Benfield, P. Hyaluronic acid. A review of its pharmacology and use as a surgical aid in ophthalmology, and its therapeutic potential in joint disease and wound healing. *Drugs* **1994**, *47*, 536–566.
28. Toole, B.P. Hyaluronan and its binding proteins, the hyaladherins. *Curr. Opin. Cell Biol.* **1990**, *2*, 839–844.
29. Tan, H.; Wu, J.; Lao, L.; Gao, C. Gelatin/chitosan/hyaluronan scaffold integrated with PLGA microspheres for cartilage tissue engineering. *Acta Biomater.* **2009**, *5*, 328–337.
30. Fraser, J.R.; Laurent, T.C.; Laurent, U.B. Hyaluronan: its nature, distribution, functions and turnover. *J. Intern. Med.* **1997**, *242*, 27–33.
31. Dowthwaite, G.P.; Edwards, J.C.; Pitsillides, A.A. An essential role for the interaction between hyaluronan and hyaluronan binding proteins during joint development. *J. Histochem. Cytochem.* **1998**, *46*, 641–651.
32. Cheung, W.F.; Crue, T.F.; Turley, E.A. Receptor for hyaluronan mediated motility (RHAMM), a hyaladherin that regulates cell responses to growth factors. *Biochem. Soc. Trans.* **1999**, *27*, 135–142.
33. Entwistle, J.; Hall, C.L.; Turley, E.A. Hyaluronan receptors: regulators of signalling to the cytoskeleton. *J. Cell Biochem.* **1996**, *61*, 569–577.
34. Liu, L.S.; Thompson, A.Y.; Heidaran, M.A.; Poser, J.W.; Spiro, R.C. An osteoconductive collagen/hyaluronate matrix for bone regeneration. *Biomaterials* **1999**, *20*, 1097–1108.
35. Campoccia, D.; Doherty, P.; Radice, M.; Brun, P.; Abatangelo, G.; Williams, D.F. Semisynthetic resorbable materials from hyaluronan esterification. *Biomaterials* **1998**, *23*, 2101–2127.
36. Rowley, J.A.; Madlambayan, G.; Mooney, D.J. Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials* **1999**, *20*, 45–53.
37. Lee, K.Y.; Kong, H.J.; Larson, R.G.; Mooney, D.J. Hydrogel formation *via* cell crosslinking. *Adv. Mater.* **2003**, *15*, 1828–1832.
38. Cao, Y.; Shen, X.C.; Chen, Y.; Guo, J.; Chen, Q.; Jiang, X.Q. pH-Induced self-assembly and capsules of sodium alginate. *Biomacromolecules* **2005**, *6*, 2189–2196.
39. Paige, K.T.; Cima, L.G.; Yaremchuk, M.J.; Vacanti, J.P.; Vacanti, C.A. Injectable cartilage. *Plast. Reconstr. Surg.* **1995**, *96*, 1390–1400.

40. Paige, K.T.; Cima, L.G.; Yaremchuk, M.J.; Schloo, B.L.; Vacanti, J.P.; Vacanti, C.A. De novo cartilage generation using calcium alginate-chondrocyte constructs. *Plast. Reconstr. Surg.* **1996**, *97*, 168–180.
41. Skjak-Braerk, G.; Grasdalen, H.; Smidsrod, O. Inhomogeneous polysaccharide ionic gels. *Carbohydr. Polym.* **1989**, *10*, 31–54.
42. Stevens, M.M.; Qanadilo, H.F.; Langer, R.; Shastri, V.P. A rapid-curing alginate gel system: Utility in periosteum-derived cartilage tissue engineering. *Biomaterials* **2004**, *25*, 887–894.
43. Kuo, C.K.; Ma, P.X. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. *Biomaterials* **2001**, *22*, 511–521.
44. Bouhadir, K.H.; Lee, K.Y.; Alsberg, E.; Damm, K.L.; Anderson, K.W.; Mooney, D.J. Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnol. Prog.* **2001**, *17*, 945–950.
45. Kong, H.J.; Alsberg, E.; Kaigler, D.; Lee, K.Y.; Mooney, D.J. Controlling degradation of hydrogel *via* the size of cross-linked junctions. *Adv. Mater.* **2004**, *16*, 1917–1921.
46. Balakrishnan, B.; Jayakrishnan, A. Self-cross-linking biopolymers as injectable *in situ* forming biodegradable scaffolds. *Biomaterials* **2005**, *26*, 3941–3951.
47. Behraves, E.; Sikavitsas, V.I.; Mikos, A.G. Quantification of ligand surface concentration of bulk-modified biomimetic hydrogels. *Biomaterials* **2003**, *24*, 4365–4374.
48. Anseth, K.S.; Metters, A.T.; Bryant, S.J.; Martens, P.J.; Elisseff, J.H.; Bowman, C.N. *In situ* forming degradable networks and their application in tissue engineering and drug delivery. *J. Control. Rel.* **2002**, *78*, 199–209.
49. Guillaudeu, S.J.; Fox, M.E.; Haidar, Y.M.; Dy, E.E.; Szoka, F.C.; Fréchet, J.M.J. PEGylated dendrimers with core functionality for biological applications. *Bioconjugate. Chem.* **2008**, *19*, 461–469.
50. Feng, X.; Taton, D.; Chaikof, E.L.; Gnanou, Y. Bouquet-type dendrimer-like poly(ethylene Oxide)s with a focal aldehyde and peripheral hydroxyls. *Biomacromolecules* **2007**, *8*, 2374–2378.
51. Hiemstra, C.; Zhong, Z.; Li, L.; Dijkstra, P.J.; Feijen, J. *In-situ* formation of biodegradable hydrogels by stereocomplexation of PEG-(PLLA)₈ and PEG-(PDLA)₈ star block copolymers. *Biomacromolecules* **2006**, *7*, 2790–2795.
52. Hiemstra, C.; Zhong, Z.; van Tomme, S.R.; van Steenberg, M.J.; Jacobs, J.J.L.; Otter, W.D.; Hennink, W.E.; Feijen, J. *In vitro* and *in vivo* protein delivery from *in situ* forming poly(ethylene glycol)–poly(lactide) hydrogels. *J. Control. Rel.* **2007**, *119*, 320–327.
53. Holland, T.A.; Tessmar, J.K.; Tabata, Y.; Mikos, A.G. Transforming growth factor-beta1 release from oligo(poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. *J. Control. Rel.* **2004**, *94*, 101–114.
54. Wieland, J.A.; Houchin-Ray, T.L.; Shea, L.D. Non-viral vector delivery from PEG-hyaluronic acid hydrogels. *J. Control. Rel.* **2007**, *120*, 233–241.
55. Leach, J.B.; Bivens, K.A.; Collins, C.N.; Schmidt, C.E. Development of photocrosslinkable hyaluronic acid polyethylene glycol-peptide composite hydrogels for soft tissue engineering. *J. Biomed. Mater. Res.* **2004**, *70A*, 74–82.

56. Almany, L.; Seliktar, D. Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures. *Biomaterials* **2005**, *26*, 2467–2477.
57. Bhattarai, N.; Ramay, H.R.; Gunn, J.; Matsen, F.A.; Zhang, M. PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. *J. Control. Rel.* **2005**, *103*, 609–624.
58. Yamaguchi, N.; Chae, B.S.; Zhang, L.; Kiick, K.L.; Furst, E.M. Rheological characterization of polysaccharide-poly(ethylene glycol) star copolymer hydrogels. *Biomacromolecules* **2005**, *6*, 1931–1940.
59. Nicodemus, G.D.; Villanueva, I.; Bryant, S.J. Mechanical stimulation of TMJ condylar chondrocytes encapsulated in PEG hydrogels. *J. Biomed. Mater. Res.* **2007**, *83A*, 323–331.
60. Hudalla, G.A.; Eng, T.S.; Murphy, W.L. An approach to modulate degradation and mesenchymal stem cell behavior in poly(ethylene glycol) networks. *Biomacromolecules* **2008**, *9*, 842–849.
61. Weber, L.M.; Cheung, C.Y.; Anseth, K.S. Multifunctional pancreatic islet encapsulation barriers achieved via multilayer PEG hydrogels. *Cell Transplant.* **2008**, *16*, 1049–1057.
62. Brown, C.D.; Stayton, P.S.; Hoffman, A.S. Semi-interpenetrating network of poly(ethylene glycol) and poly(D,L-lactide) for the controlled delivery of protein drugs. *J. Biomater. Sci. Polym. Edn.* **2005**, *16*, 189–201.
63. Cascone, M.G.; Laus, M.; Ricci, D.; Sbarbati Del Guerra, R. Evaluation of poly(vinyl alcohol) hydrogels as a component of hybrid artificial tissues. *J. Mater. Sci.: Mater. Med.* **1995**, *6*, 71–75.
64. Nuttelman, C.R.; Mortisen, D.J.; Henry, S.M.; Anseth, K.S. Attachment of fibronectin to poly(vinyl alcohol) hydrogels promotes NIH3T3 cell adhesion, proliferation, and migration. *J. Biomed. Mater. Res.* **2001**, *57*, 217–223.
65. Chenite, A.; Chaput, C.; Wang, D.; Combes, C.; Buschmann, M.D.; Hoemann, C.D.; Leroux, J.C.; Atkinson, B.L.; Binette, F.; Selmani, A. Novel injectable neutral solutions of chitosan from biodegradable gels *in situ*. *Biomaterials* **2000**, *21*, 2155–2161.
66. Chenite, A.; Buschmann, M.; Wang, D.; Chaput, C.; Kandani, N. Rheological characterisation of thermogelling chitosan/glycerol-phosphate solutions. *Carbohydr. Polym.* **2001**, *46*, 39–46.
67. Crompton, K.E.; Prankerd, R.J.; Paganin, D.M.; Scott, T.F.; Horne, M.K.; Finkelstein, D.I.; Gross, K.A.; Forsythe, J.S. Morphology and gelation of thermosensitive chitosan hydrogels. *Biophys. Chem.* **2005**, *117*, 47–53.
68. Hoemann, C.D.; Sun, J.; Legare, A.; McKee, M.D.; Buschmann, M.D. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. *Osteoarthritis Cartilage* **2005**, *13*, 318–329.
69. Shimizu, T.; Yamato, M.; Isoi, Y.; Akutsu, T.; Setomaru, T.; Abe, K.; Kikuchi, A.; Umezumi, M.; Okano, T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ. Res.* **2002**, *90*, 40–48.
70. Gan, T.; Zhang, Y.; Guan, Y. *In situ* gelation of P(NIPAM-HEMA) microgel dispersion and its applications as injectable 3D cell scaffold. *Biomacromolecules* **2009**, *10*, 1410–1415.
71. Kim, J.H.; Lee, S.S.; Kim, S.J.; Lee, Y.M. Rapid temperature/pH response of porous alginate-g-poly(*N*-isopropylacrylamide) hydrogels. *Polymer* **2002**, *43*, 7549–7558.

72. Wang, L.Q.; Tu, K.; Li, Y.; Zhang, J.; Jiang, L.; Zhang, Z. Synthesis and characterization of temperature responsive graft copolymers of dextran with poly(*N*-isopropylacrylamide). *React Funct. Polym.* **2002**, *53*, 19–27.
73. Lin, H.H.; Cheng, Y.L. *In-situ* thermoreversible gelation of block and star copolymers of poly(ethylene glycol) and poly(*N*-isopropylacrylamide) of varying architectures. *Macromolecules* **2001**, *34*, 3710–3715.
74. Stile, R.A.; Burghardt, W.R.; Healy, K.E. Synthesis and characterization of injectable poly(*N*-isopropylacrylamide)-based hydrogels that support tissue formation *in vitro*. *Macromolecules* **1999**, *32*, 7370–7379.
75. Lee, S.B.; Ha, D.I.; Cho, S.K.; Kim, S.J.; Lee, Y.M. Temperature/pH-sensitive comb-type graft hydrogels composed of chitosan and poly(*N*-isopropylacrylamide). *J. Appl. Polym. Sci.* **2004**, *92*, 2612–2620.
76. Lee, J.W.; Jung, M.C.; Park, H.D.; Park, K.D.; Ryu, G.H. Synthesis and characterization of thermosensitive chitosan copolymer as a novel biomaterial. *J. Biomed. Mater. Res.* **2004**, *15*, 1065–1079.
77. Wang, J.; Chen, L.; Zhao, Y.; Guo, G.; Zhang, R. Cell adhesion and accelerated detachment on the surface of temperature-sensitive chitosan and poly(*N*-isopropylacrylamide) hydrogels. *J. Mater. Sci. Mater. Med.* **2009**, *20*, 583–590.
78. Chen, J.P.; Cheng, T.H. Thermo-responsive chitosan-graft-poly(*N*-isopropylacrylamide) injectable hydrogel for cultivation of chondrocytes and meniscus cells. *Macromol. Biosci.* **2006**, *6*, 1026–1039.
79. Tan, H.; Ramirez, C.M.; Miljkovic, N.; Li, H.; Rubin, J.P.; Marra, K.G. Thermosensitive Injectable Hyaluronic Acid Hydrogel for Adipose Tissue Engineering. *Biomaterials* **2009**, *30*, 6844–6853.
80. Cho, J.H.; Kim, S.H.; Park, K.D.; Jung, M.C.; Yang, W.I.; Han, S.W.; Noh, J.Y.; Jin, J.W.; Lee, W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(*N*-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials* **2004**, *25*, 5743–5751.
81. Ha, D.I.; Lee, S.B.; Chong, M.S.; Lee, Y.M.; Kim, S.Y.; Park, Y.H. Preparation of thermo-responsive and injectable hydrogels based on hyaluronic acid and poly(*N*-isopropylacrylamide) and their drug release behaviors. *Macromol. Res.* **2006**, *14*, 87–93.
82. Guan, J.; Hong, Y.; Ma, Z.; Wagner, W. Protein-reactive, thermoresponsive copolymers with high flexibility and biodegradability. *Biomacromolecules* **2008**, *9*, 1283–1292.
83. Ohya, S.; Nakayama, Y.; Matsuda, T. Thermoresponsive artificial extracellular matrix for tissue engineering: hyaluronic acid bioconjugated with poly(*N*-isopropylacrylamide) grafts. *Biomacromolecules* **2001**, *2*, 856–863.
84. Ibusuki, S.; Fujii, Y.; Iwamoto, Y.; Matsuda, T. Tissue-engineered cartilage using an injectable and *in situ* gelable thermoresponsive gelatin: fabrication and *in vitro* performance. *Tissue Eng.* **2003**, *9*, 371–384.
85. Bogdanov, B.; Vidts, A.; Bulcke, A.; Verbeeck, R.; Schacht, E. Synthesis and thermal properties of poly(ethylene glycol)-poly(ϵ -caprolactone) copolymers. *Polymer* **1998**, *39*, 1631–1636.

86. Shim, W.S.; Kim, J.-H.; Park, H.; Kim, K.; Kwon, I.C.; Lee, D.S. Biodegradability and biocompatibility of a pH- and thermo-sensitive hydrogel formed from a sulfonamide-modified poly(ϵ -caprolactone-co-lactide)–poly(ethylene glycol)–poly(ϵ -caprolactone-co-lactide) block copolymer. *Biomaterials* **2006**, *27*, 5178–5185.
87. Cellesi, F.; Tirelli, N.; Hubbell, J.A. Materials for cell encapsulation via a new tandem approach combining reverse thermal gelation and covalent crosslinking. *Macromol. Chem. Phys.* **2002**, *203*, 1466–1472.
88. Jeong, B.; Bae, Y.H.; Kim, S.W. *In situ* gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *J. Biomed. Mater. Res.* **2000**, *50*, 171–177.
89. Sieminski, A.L.; Semino, C.E.; Gong, H.; Kamm, R.D. Primary sequence of ionic self-assembling peptide gels affects endothelial cell adhesion and capillary morphogenesis. *J. Biomed. Mater. Res. A* **2008**, *87*, 494–504.
90. Paramonov, S.E.; Jun, H.W.; Hartgerink, J.D. Modulation of peptide-amphiphile nanofibers via phospholipid inclusions. *Biomacromolecules* **2006**, *7*, 24–6.
91. Murakami, Y.; Maeda, M. DNA-responsive hydrogels that can shrink or swell. *Biomacromolecules* **2005**, *6*, 2927–2929.
92. Elisseeff, J.; McIntosh, W.; Anseth, K.; Riley, S.; Ragan, P.; Langer, R. Photoencapsulation of chondrocytes in poly(ethylene oxide)-based semi-interpenetrating networks. *J. Biomed. Mater. Res.* **2000**, *51*, 164–171.
93. Varghese, S.; Hwang, N.S.; Canver, A.C.; Theprungsirikul, P.; Lin, D.W.; Elisseeff, J. Chondroitin sulfate based niches for chondrogenic differentiation of mesenchymal stem cells. *Matrix Biology* **2008**, *27*, 12–21.
94. Park, Y.D.; Tirelli, N.; Hubbell, J.A. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. *Biomaterials* **2003**, *24*, 893–900.
95. Masters, K.S.; Shah, D.N.; Walker, G.; Leinwand, L.A.; Anseth, K.S. Designing scaffolds for valvular interstitial cells: cell adhesion and function on naturally derived materials. *J. Biomed. Mater. Res.* **2004**, *71A*, 172–180.
96. Leach, J.B.; Bivens, K.A.; Collins, C.N.; Schmidt, C.E. Development of photocrosslinkable hyaluronic acid polyethylene glycol-peptide composite hydrogels for soft tissue engineering. *J. Biomed. Mater. Res.* **2004**, *70A*, 74–82.
97. Jongpaiboonkit, L.; King, W.J.; Lyons, G.E.; Paguirigan, A.L.; Warrick, J.W.; Beebe, D.J.; Murphy, W.L. An adaptable hydrogel array format for 3-dimensional cell culture and analysis. *Biomaterials* **2008**, *29*, 3346–3356.
98. DeLong, S.A.; Gobin, A.S.; West, J.L. Covalent immobilization of RGDS on hydrogel surfaces to direct cell alignment and migration. *J. Control. Rel.* **2005**, *109*, 139–148.
99. Garagorri, N.; Fermanian, S.; Thibault, R.; Ambrose, W.M.; Schein, O.D.; Chakravarti, S.; Elisseeff, J. Keratocyte behavior in three-dimensional photopolymerizable poly(ethylene glycol) hydrogels. *Acta Biomater.* **2008**, *4*, 1139–1147.
100. Bryant, S.J.; Anseth, K.S.; Lee, D.A.; Bader, D.L. Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain. *J. Orthop. Res.* **2004**, *22*, 1143–1149.

101. Rice, M.A.; Anseth, K.S. Encapsulating chondrocytes in copolymer gels: Bimodal degradation kinetics influence cell phenotype and extracellular matrix development. *J. Biomed. Mater. Res.* **2004**, *70A*, 560–568.
102. Bryant, S.J.; Bender, R.; Durand, K.L.; Anseth, K.S. Encapsulating chondrocytes in degrading PEG hydrogels with high modulus: engineering gel structural changes to facilitate cartilaginous tissue production. *Biotechnol. Bioeng.* **2004**, *86*, 747–755
103. Hu, X.; Gao, C. Photoinitiating polymerization to prepare biocompatible chitosan hydrogels. *J. Appl. Polym. Sci.* **2008**, *110*, 1059–1067.
104. Hong, Y.; Mao, Z.; Wang, H.; Gao, C.; Shen, J. Covalently crosslinked chitosan hydrogel formed at neutral pH and body Temperature. *J. Biomed. Mater. Res.* **2006**, *79A*, 913–922.
105. Peter, S.J.; Yaszemski, M.J.; Suggs, L.J.; Payne, R.G.; Hayes, W.C.; Langer, R.; Unroe, M.; Alemany, L.B.; Engel, P.S.; Mikos, A.G. Characterization of partially saturated poly(propylene fumarate) for orthopaedic application. *J. Biomater. Sci. Polym. Edn.* **1997**, *8*, 893–904.
106. Peter, S.J.; Lu, L.C.; Mikos, A.G. Marrow stromal osteoblast function on a poly(propylene fumarate)/ β -tricalcium phosphate biodegradable orthopaedic composite. *Biomaterials* **2000**, *21*, 1207–1213.
107. He, S.L.; Yaszemski, M.J.; Yasko, A.W.; Engel, P.S.; Mikos, A.G. Injectable biodegradable polymer composites based on poly(propylene fumarate) crosslinked with poly(ethylene glycol)-dimethacrylate. *Biomaterials* **2000**, *21*, 2389–2394.
108. Peter, S.J.; Miller, S.T.; Zhu, G.M.; Yasko, A.W.; Mikos, A.G. *In vivo* degradation of a poly(propylene fumarate)/ β -tricalcium phosphate injectable composite scaffold. *J. Biomed. Mater. Res.* **1998**, *41*, 1–7.
109. Shin, H.; Ruhé, P.Q.; Mikos, A.G.; Jansen, J.A. *In vivo* bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. *Biomaterials* **2003**, *24*, 3201–3211.
110. Jo, S.; Shin, H.; Shung, A.K.; Fisher, J.P.; Mikos, A.G. Synthesis and characterization of oligo(poly(ethylene glycol) fumarate) macromer. *Macromolecules* **2001**, *34*, 2839–2844.
111. Temenoff, J.S.; Park, H.; Jabbari, E.; Sheffield, T.L.; LeBaron, R.G.; Ambrose, C.G.; Mikos, A.G. *In vitro* osteogenic differentiation of marrow stromal cells encapsulated in biodegradable hydrogels. *J. Biomed. Mater. Res.* **2004**, *70A*, 235–244.
112. Lutolf, M.P.; Tirelli, N.; Cerritelli, S.; Cavalli, L.; Hubbell, J.A. Systematic modulation of Michael-type reactivity of thiols through the use of charged amino acids. *Bioconjugate Chem.* **2001**, *12*, 1051–1056.
113. Park, Y.D.; Tirelli, N.; Hubbell, J.A. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. *Biomaterials* **2003**, *24*, 893–900.
114. Pratt, A.B.; Weber, F.E.; Schmoekel, H.G.; Müller, R.; Hubbell, J.A. Synthetic extracellular matrices for *in situ* tissue engineering. *Biotechnol. Bioeng.* **2004**, *86*, 27 – 36.
115. Vernon, B.; Tirelli, N.; Bächli, T.; Haldimann, D.; Hubbell, J.A. Water-borne, *in situ* crosslinked biomaterials from phase-segregated precursors. *J. Biomed. Mater. Res.* **2003**, *64A*, 447–456.
116. Lutolf, M.P.; Hubbell, J.A. Synthesis and physicochemical characterization of end-linked poly(ethylene glycol)-co-peptide hydrogels formed by Michael-type addition. *Biomacromolecules* **2003**, *4*, 713–722.

117. Lutolf, M.P.; Raeber, G.P.; Zisch, A.H.; Nicola, T.; Hubbell J.A. Cell-responsive synthetic hydrogels. *Adv. Mater.* **2003**, *15*, 888–892.
118. Sung, H.W.; Huang, R.N.; Huang, L.L.H.; Tsai, C.C.; Chiu, C.T. Feasibility study of a natural crosslinking reagent for biological tissue fixation. *J. Biomed. Mater. Res.* **1998**, *42*, 560–567.
119. Chang, Y.; Tsai, C.C.; Liang, H.C.; Sung, H.W. *In vivo* evaluation of cellular and acellular bovine pericardia fixed with a naturally occurring crosslinking agent (genipin). *Biomaterials* **2002**, *23*, 2447–2457.
120. Tsai, C.C.; Huang, R.N.; Sung, H.W.; Liang, H.C. *In vitro* evaluation of the genotoxicity of a naturally occurring crosslinking agent (genipin) for biologic tissue fixation. *J. Biomed. Mater. Res.* **2000**, *52*, 58–65.
121. Liu, B.S.; Yao, C.H.; Chen, Y.S.; Hsu, S.H. *In vitro* evaluation of degradation and cytotoxicity of a novel composite as a bone substitute. *J. Biomed. Mater. Res.* **2003**, *67A*, 1163–1169.
122. Mi, F.L.; Tan, Y.C.; Liang, H.C.; Huang, R.N.; Sung, H.W. *In vitro* evaluation of a chitosan membrane cross-linked with genipin. *J. Biomater. Sci. Polym. Ed.* **2001**, *12*, 835–850.
123. Butler, M.F.; Yiu-Fai, N.G.; Pudney, D.A. Mechanism and kinetics of crosslinking reaction between biopolymers containing primary amine groups and genipin. *J. Polym. Sci. A* **2003**, *41*, 3941–3953.
124. Moffat, K.L.; Marra, K.G. Biodegradable poly(ethylene glycol) hydrogels crosslinked with genipin for tissue engineering applications. *J. Biomed. Mater. Res.* **2004**, *71B*, 181–187.
125. Ferretti, M.; Marra, K.G.; Kobayashi, K.; Defail, A.J.; Chu, C.R. Controlled *in vivo* degradation of genipin crosslinked polyethylene glycol hydrogels within osteochondral defects. *Tissue Eng.* **2006**, *12*, 2657–2663.
126. DeFail, A.J.; Chu, C.R.; Izzo, N.; Marra, K.G. Controlled release of bioactive TGF- β 1 from microspheres embedded within biodegradable hydrogels. *Biomaterials* **2006**, *27*, 1579–1585.
127. Tan, H.; DeFail, A.J.; Rubin, J.P.; Chu, C.R.; Marra, K.G. Novel multi-arm PEG-based hydrogels for tissue engineering. *J. Biomed. Mater. Res.* **2010**, *92A*, 979–987.
128. Tan, H.; Chu, C.R.; Payne, K.A.; Marra, K.G. Injectable *in situ* forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials* **2009**, *30*, 2499–2506.
129. Maia, J.; Ferreira, L.; Carvalho, R.; Ramos, M.A.; Gil, M.H. Synthesis and characterization of new injectable and degradable dextran-based hydrogels. *Polymer* **2005**, *46*, 9604–9614.
130. Nishi, K.K.; Jayakrishnan, A. Preparation and *in vitro* evaluation of primaquine-conjugated gum arabic microspheres. *Biomacromolecules* **2004**, *5*, 1489–1495.
131. Wang, D.A.; Varghese, S.; Sharma, B.; Strehin, I.; Fermanian, S.; Gorham, J.; Fairbrother, D.H.; Cascio, B.; Elisseeff, J.H. Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration. *Nat. Mater.* **2007**, *6*, 385–392.
132. Ruhela, D.; Riviere, K.; Szoka, F.C. Efficient synthesis of an aldehyde functionalized hyaluronic acid and its application in the preparation of hyaluronan-lipid conjugates. *Bioconjug. Chem.* **2006**, *17*, 1360–1363.
133. Ito, T.; Yeo, Y.; Highley, C.B.; Bellas, E.; Benitez, C.A.; Kohane, D.S. The prevention of peritoneal adhesions by *in situ* cross-linking hydrogels of hyaluronic acid and cellulose derivatives. *Biomaterials* **2007**, *28*, 975–983.

134. Jia, X.; Yeo, Y.; Clifton, R.J.; Jiao, T.; Kohane, D.S.; Kobler, J.B.; Zeitels, S.M.; Langer, R. Hyaluronic acid-based microgels and microgel networks for vocal fold regeneration. *Biomacromolecules* **2006**, *7*, 3336–3344.
135. Holland, T.A.; Tessmar, J.K.; Tabata, Y.; Mikos, A.G. Transforming growth factor-beta 1 release from oligo(poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. *J. Control. Rel.* **2004**, *94*, 101–114.
136. Ferretti, M.; Marra, K.G.; Kobayashi, K.; DeFail, A.J.; Chu, C.R. Controlled *in vivo* degradation of genipin crosslinked poly(ethylene glycol) hydrogels within osteochondral defects. *Tissue Eng.* **2006**, *12*, 2657–2663.
137. Hu, Y.; Hollinger, J.O.; Marra, K.G. Controlled release from coated polymer microparticles embedded in tissue-engineered scaffolds. *J. Drug Targeting* **2001**, *9*, 431–438.
138. Marra, K.G.; Defail, A.J.; Clavijo-Alvarez, J.A.; Badylak, S.F.; Taieb, A.; Schipper, B.; Bennett, J.; Rubin, J.P. FGF-2 enhances vascularization for adipose tissue engineering. *Plast. Reconstr. Surg.* **2008**, *121*, 1153–1164.
139. Rubin, J.P.; Bennett, J.M.; Doctor, J.S.; Tebbets, B.M.; Marra, K.G. Collagenous microbeads as a scaffold for tissue engineering with adipose-derived stem cells. *Plast. Reconstr. Surg.* **2007**, *120*, 414–424.
140. Cooper, C.; Snow, S.; McAlindon, T.E.; Kellingray, S.; Stuart, B.; Coggon, D. Risk factors for the incidence and progression of radiographic knee osteoarthritis. *Arthritis Rheum* **2000**, *43*, 995–1000.
141. Lawrence, R.; Helmick, C.; Arnett, F.; Deyo, R.; Felson, D.; Giannini, E.; Heyse, S.; Hirsch, R.; Hochberg, M.; Hunder, G.; Liang, M.; Pillemer, S.; Steen, V.; Wolfe, F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* **1998**, *41*, 778–799.
142. Finch, G.D.; Dawe, C.J. Hemiatrophy. *J. Pediatr. Orthop.* **2003**, *23*, 99–101.
143. Fokin, A.A.; Robicsek, F. Poland's syndrome revisited. *Ann. Thorac. Surg.* **2002**, *74*, 2218–2225.
144. Peer, L.A. The neglected free fat graft. *Plast. Reconstr. Surg.* **1956**, *18*, 233–250.
145. Hemmrich, K.; Van de Sijpe, K.; Rhodes, N.P.; Hunt, J.A.; Di Bartolo, C.; Pallua, N.; Blondeel, P.; von Heimburg, D. Autologous *in vivo* adipose tissue engineering in hyaluronan—based gels—a pilot study. *J. Surg. Res.* **2008**, *144*, 82–88.
146. West, D.C.; Kumar, S. Hyaluronan and angiogenesis. *Ciba. Found Symp.* **1989**, *143*, 187–201.
147. Yuksel, E.; Weinfeld, A.B.; Cleek, R.; Wamsley, S.; Jensen, J.; Boutros, S.; Waugh, J.M.; Shenaq, S.M.; Spira, M. Increased free fat-graft survival with the long-term, local delivery of insulin, insulin-like growth factor-i, and basic fibroblast growth factor by plga/peg microspheres. *Plast. Reconstr. Surg.* **2000**, *105*, 1712–1720.
148. Kimura, Y.; Ozeki, M.; Inamoto, T.; Tabata, Y. Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor. *Biomaterials* **2003**, *2324*, 2513–2521.