Chapter 7
Part III: Tissue Engineering

7.1 Introduction

Hyperbranched polymers have a three-dimensional structure with high functionality, high reactivity due to the presence of a large number of free terminal groups, and they exhibit enhanced absorption capacity of biomolecules on a polymeric biomaterial. More advantage with these architectural polymers is that they can be altered structurally as well as by incorporation of functional groups can be improved for better cell attachment. Hyperbranched polymers are quite capable of forming porous hydrogels or films as scaffolds, and are promising material to support adhesion and rapid reproduction of cells. Thus, hyperbranched polymers, due to their unique structures and special properties, have proved to be of high potential in various applications in tissue engineering fields.

7.2 Hyperbranched Polymers as Tissue Scaffold Component

A wide variety of hyperbranched polymers, such as hyperbranched poly(lactic acid) (PLA), poly(lactic-glycolic acid) (PLGA), polycaprolactone (PCL), polyurethane, polyethylene glycol (PEG), polyglycerol (PG), poly(NIPAM) have been extensively examined and widely studied for fabrication of tissue engineering scaffolds [1–6].

With high number of functional end groups, biocompatible hyperbranched polymers provide a more densely packed matrix and has proved to be suitable as an ideal biological scaffold with the following characteristics: (1) it possesses a porous three-dimensional network that is resolvable in vivo, (2) it is comparable with the mechanical properties of the normal human cartilage, (3) it promotes cell growth in the surrounding joint area, (4) it does not alter the immunity system of the body,

A. Bandyopadhyay et al., *Hyperbranched Polymers for Biomedical Applications*,
Springer Series on Polymer and Composite Materials,
https://doi.org/10.1007/978-981-10-6514-9_7

(5) it bears high strength which can be placed in joint area and it is able to withstand the physiological loads until the tissue repair is complete, (6) it resists excess swelling of the matrix [7].

Due to the above-mentioned advantages provided by hyperbranched polymers, it is more preferred as tissue scaffolds. First, we will consider polyurethane-based tissue scaffolds. Polyurethanes with a biodegradable linear aliphatic polyester part and as its copolymers possess high molecular weight along with high glass transition temperature and modulus. The main advantage with HPU-based biomaterials is their mechanical strength, flexibility, and chemical and biological properties. Thus, they find a wide range of application in the biomedical field—from cardiovascular repair, cartilage implant, ligament regeneration, and bone replacement to controlled drug/gene delivery but mainly considered for hard-tissue scaffolds [8].

Karak and co-workers synthesized sunflower oil-based HPUs with different weight percentages of the branching agent, pentaerythritol. It was a remarkable research work as this was the first time that vegetable oil-based HBPs were prepared and it was examined as a potential scaffold material for tissue engineering [9]. The MTT–hemolytic assay and subcutaneous implantation in Wistar rats followed by cytokine–ALP assay and histopathology studies confirmed a better biocompatibility of HPU (Fig. 7.1).

Other than HBPU, aliphatic polyesters are also regarded as a good tissue scaffold component due to its easily degradable ester backbone, which breaks down easily by hydrolysis and makes adequate space for newly developing tissues. Therefore, glycolic acid (PGA), lactic acid (PLA), and their block copolymer poly (lactic-co-glycolic acid) (PLGA) are quite preferred as medical scaffolds and implants. As we already know that a hyperbranched polymer is less crystalline and has lower solution/melt viscosity. Therefore, processing is easier for hyperbranched polymers than their linear analogues with similar molecular weight. This property was utilised by Chiellini and his group. They used a branched PCL to generate a three-dimensional mesh by simple wet spinning method [10]. They further studied these meshes for regeneration of bone tissue along with their antimicrobial property. The biocompatibility of the branched PCL and the influence of the scaffold architecture on cell behavior were examined with MC-3T3 pre-osteoblast cells.

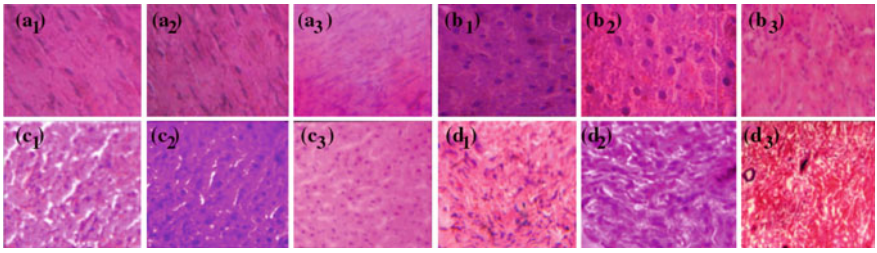


Fig. 7.1 Histological sections for heart (a_1, a_2, and a_3), for kidney (b_1, b_2, and b_3), for liver (c_1, c_2, and c_3), and for skin (d_1, d_2, and d_3) of control (1), HBPU (2), and SHBPU (3) of Wistar rats. Reprinted with permission from Ref. [9]. Copyright (2013) Wiley (Color figure online)

After 14 days of culture, from the cell adhesion and proliferation analysis it was evident that the meshes could be an alternative for the conventional materials used as engineered bone scaffolds (Fig. 7.2).

Song and his co-workers worked on amorphous shape memory polymer (SMP) network crosslinked from a star-branched macromer. It had polyhedral oligomeric silsequioxane (POSS) nanoparticle core and adjacent PLA arms [11]. The rigid POSS nanoparticle core promotes greater participation of the urethane-tethered PLA arms in the elastic deformation and the recoiling process with reduced excessive chain entanglement. Consequently, the resulting POSS-SMP nanocomposites, with cortical bone-like modulus (B2 GPa) at body temperature, could stably hold their temporary shape for more than an 1 year at room and body temperatures and achieve full shape recovery within a matter of seconds. The group further uses rat subcutaneous implantation model to examine degradation profile of these SMPs. The results showed that the degradation rates were inversely related to the length of the PLA chains within the crosslinked

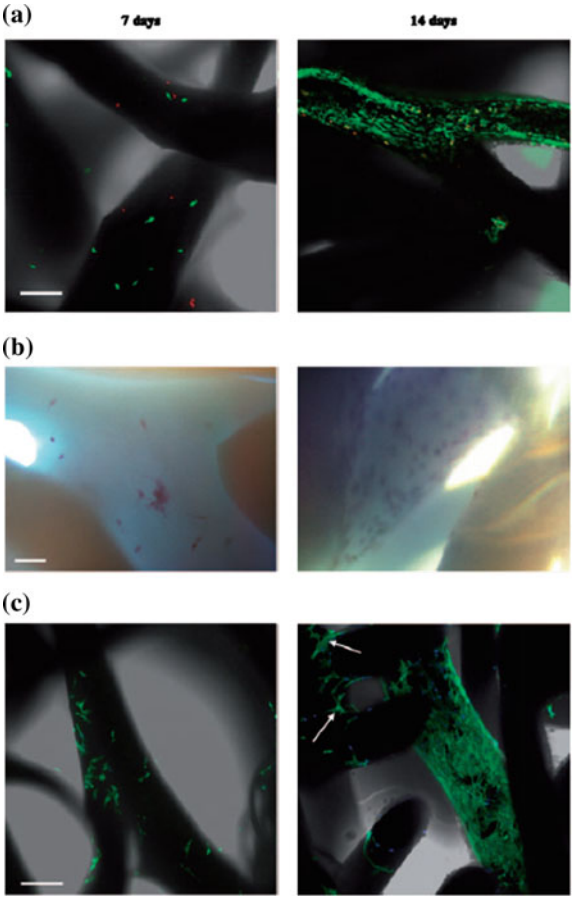


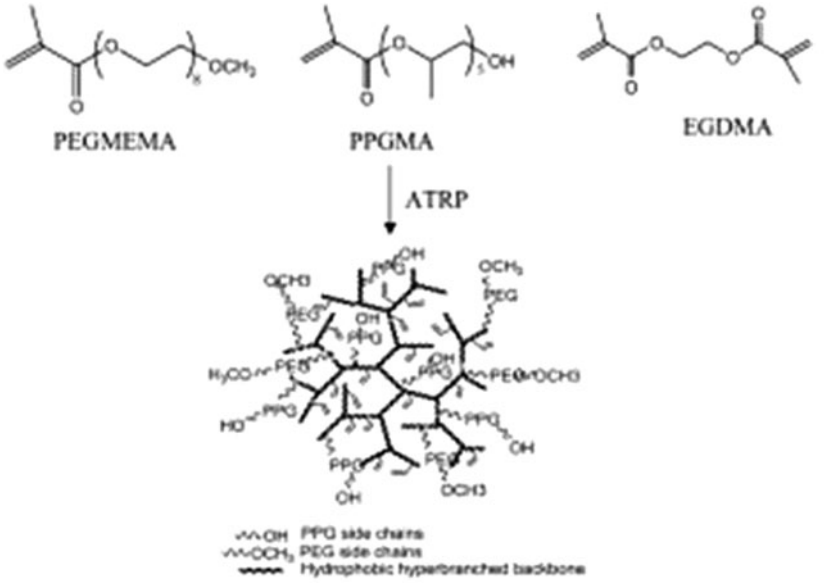
Fig. 7.2 Biological characterization of PCL meshes/MC3T3-E1 constructs. **a** Live/dead visual viability assay: viable (*green* fluorescence) and dead (*red* fluorescence) cells on scaffold. Scale bar corresponding to 200 mm, applicable to both micrographs. **b** Cytochemical analysis of the constructs: toluidine *Blue* staining shows the presence of cells on scaffold and their localization along fibers. Scale bar corresponding to 100 mm, applicable to both micrographs. **c** CLSM analysis of the constructs: actin filaments (*green*) and nuclei (*blue*) showing cellular morphology and distribution on scaffold fibers. Scale bar corresponding to 200 mm, applicable to both micrographs. Reprinted with permission from Ref. [10]. Copyright (2011) Sage publishers

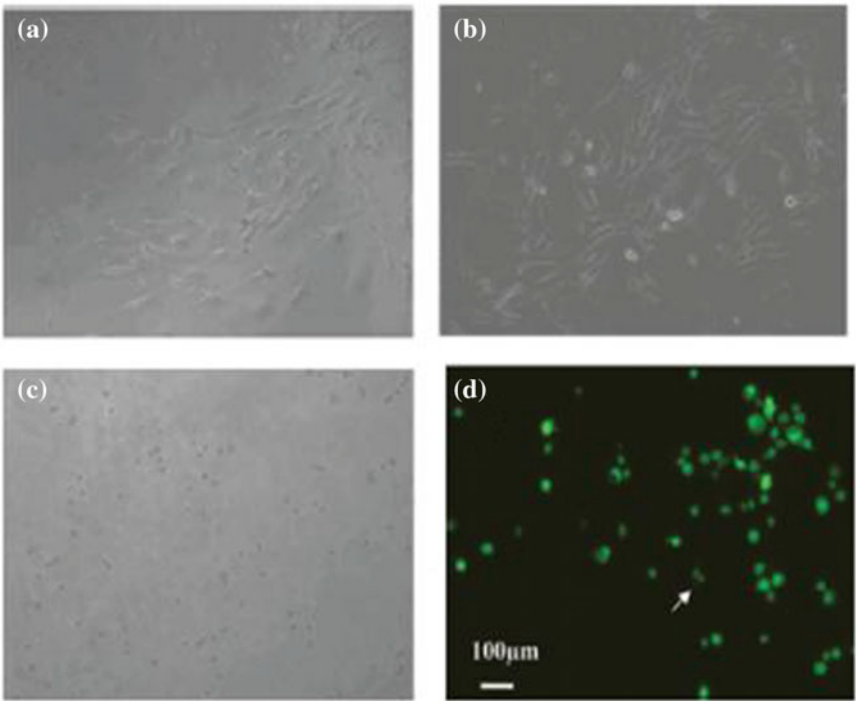
amorphous network. After 1 year of implanting these SMPs, no significant no pathologic abnormities were detected from the organs examined which makes the material ideal as a tissue scaffold material.

Hyperbranched PEGs are also considered as a potential scaffold for cell encapsulation and culture. Through various living and controlled radical polymerisation, a wide variety of hyperbranched PEGs have been developed with more controlled structure, well-defined chain lengths and with different extents of degree of branching. Lutz and his co-workers tried to modify linear poly (MEO_2MA-co-OEGMA) by introducing multifunctional vinyl monomer ethylene glycol dimethacrylate (EGDMA) within the structure [12]. They wanted to develop hyperbranched architecture but failed to do it due to microgelation formation. This method was later upgraded by Tai and his groups and they synthesized hyperbranched PEGMEMA–PPGMA–EGDMA via a one-step deactivation enhanced ATRP approach. The introduction of the multi-vinyl crosslinker EGDMA enables the copolymer with the capability of enhanced and tailorable photo-crosslinkable properties. Meanwhile, by adjusting the hydrophilic PEGMEMA and hydrophobic PPGMA composition, they can control the hydrophilicity of the polymer as the lower critical solution temperature (LCST) of the copolymer could be maintained around human body temperature [13]. The toxicity of these materials were examined over mouse C2C12 myoblast cells and they displayed very low cytotoxicity (Fig. 7.3).

Wang and his group tried to develop tissue scaffold component by varying the "long" and "short" PEG chain monomer composition and thus tried to maintain the LCST value of the copolymers around 37 °C. The 3T3 mouse fibroblast cell line was encapsulated in the hydrogel and the results were positive as there was no significant difference of cell viability between the control (cells alone) and polymer samples after 4 days of incubation. Further to improve the cytocompatibility and the cell proliferation, the authors tried to modify the structure of the polymer. They opted for thiol-modified hyaluronan biopolymer as crosslinker instead of UV crosslinking systems because of clinic safety. The hyaluronan crosslinked polymer displayed porous semi interpenetrating structure whose pore sizes and porosity varies with the polymer concentration. Thus, it becomes easier to optimize the hydrogel efficiency as tissue scaffold component (Fig. 7.4). 3T3 fibroblast cells and rabbit adipose-derived stem cells (ADSCs) were used for further biomedical studies and the results demonstrated the good cell viability after the cells were embedded inside the hydrogel. The Live/Dead assay showed that even after 1-week culture, both types of cell survived well in those three-dimensional hydrogels. The group further studied the behavior of encapsulated ADSCs and identified the secretion profile of suitable growth factors for wound healing [14].

◀**Fig. 7.3** Light phase control microscope images for the cells cultured **a** in PEGMEMA-PPGMA-EGDMA copolymer culture media solutions (750 μg/mL); **b** in the culture media without polymers, **c** on the photo-cross-linked polymer films. **d** Live/Dead viability assay for the cells cultured in the copolymer/culture media solutions after 5 days. The viable cells *fluoresce green*, whereas the nonviable cells fluoresce red (pointed by the *arrow*). Reprinted with permission from Ref. [13]. Copyright (2009) American Chemical Society (Color figure online)


PEGMEMA
PPGMA
EGDMA
ATRP
PPG side chains
PEG side chains
Hydrophobic hyperbranched backbone


(a)
(b)
(c)
(d)
100μm

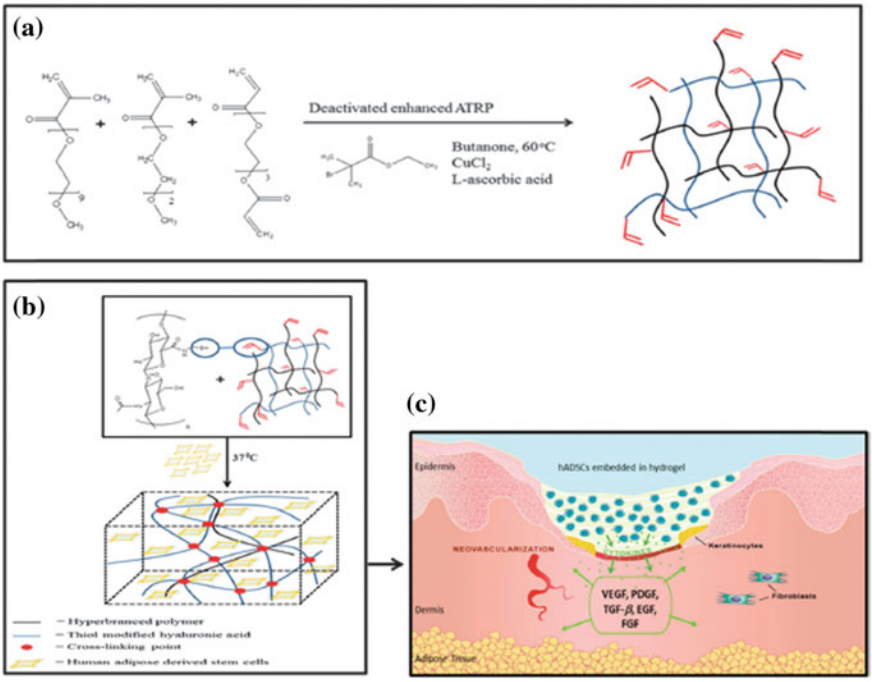


Fig. 7.4 Schematic illustration of PEGMEMA475–MEO2MA–PEGDA258 copolymer synthesis (**a**) and encapsulation of hADSCs in the crosslinked P–SH–HA hydrogel (**b**) and the cartoon picture of application of P–SH–HA hydrogel on a skin wound (**c**) for secretion of growth factors to accelerate wound healing. Reprinted with permission from Ref. [14]. Copyright (2013) Biomed Central

Large flexibility during material design is the primary advantage for the PEG-based hydrogels, but there are certain limitations that restricts the wide spread applications of these hyrogels. They do not have any definite mechanism for interacting with cells and cell adhesion is also non-specified [15]. Thus, PEG hydrogels are often modified using peptides or phosphates to promote improved cellular interactions [16, 17].

Another important subclass of polymers need to be mentioned as an important component for tissue scaffolds is hyperbranched polyglycerols (HPGs), it can be considered as a good substitute for PEG-based hydrogels due to its hydrophilicity as well as the high extent of hydroxyl functionality. Frey and his co-workers initiated the concept that HPG can be used as tissue scaffold component. They synthesized HPG hydrogels based on PEO multiarm stars with a hyperbranched dendritic core. The hydrogel products showed excellent stability with a high compression module due to its polyether backbone [18]. The efficiency of these hydrogels as substrates for cell growth has been examined and it was proved that they hold a good potential for cell growth and tissue engineering purpose.

Later, the biocompatibility of HPGs was examined by Brooks and his group using fibroblast and endothelial cells (Fig. 7.5) [19]. The assay results showed remarkably low cytotoxicity of HPG against both the cell lines.

Derivatives of HPG were also studied for tissue engineering applications. The structure was modified by attaching hydrophobic C18 alkyl chains as well as PEG-350 chains to a certain fraction of the polyether polyol OH groups [20]. Due to high solubility and low viscosity of these hyperbranched polymers, along with easy synthetic approach, they are considered as human serum albumin (HSA) substitutes. They provide a greater advantage over native HSA where there is risk of transmission of diseases. Plasma half-lives as high as 34 h as well no risk of any disease transmission makes these modified HPG suitable as synthetic plasma expanders (Fig. 7.6).

Hennink and co-workers developed dimensionally stable networks from HPG by modifying the end hydroxyl group of the polymer HPG with the photo-crosslinkable acrylate. The HPGs showed low swelling capability—thus the three-dimensional networks were highly stable. A number of sets were prepared by varying the degree of substitution and the properties of the HPG could be altered by

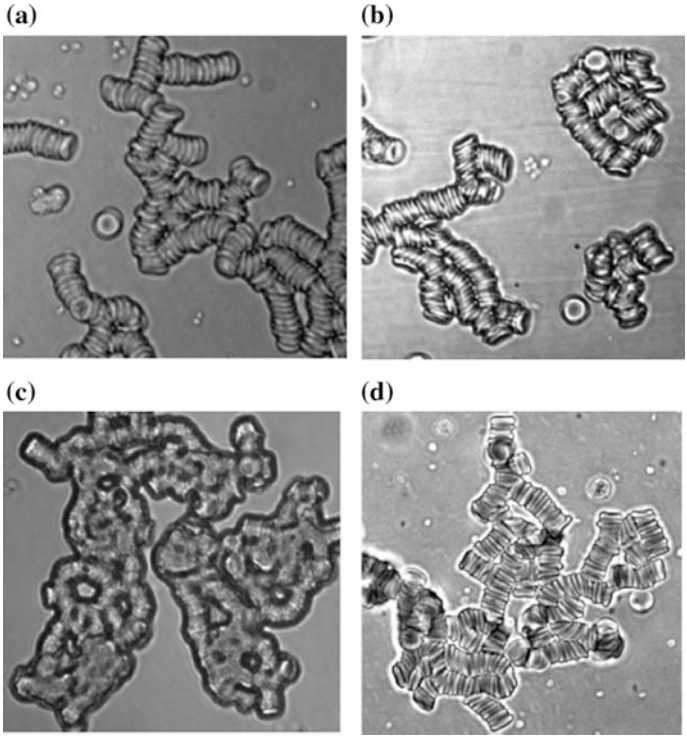


Fig. 7.5 Images of human blood red cells in anticoagulated plasma after 2 h incubation with **a** HPG, **b** LPG, **c** hetastarch, and **d** saline. Reprinted with permission from Ref. [19]. Copyright (2006) American Chemical Society

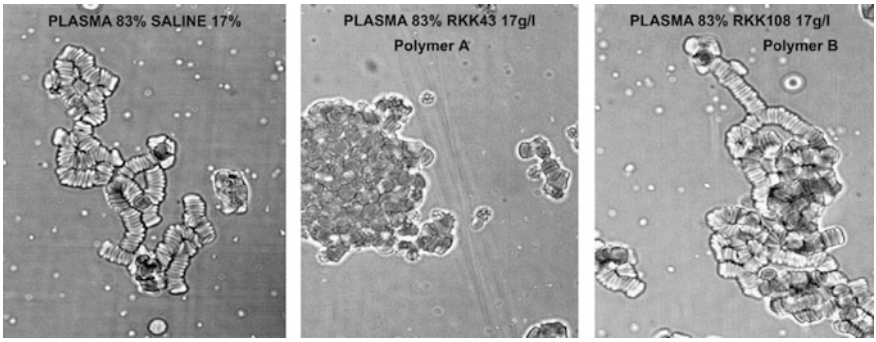


Fig. 7.6 Effect of dHPG polymers on red cells suspended in partially diluted plasma. Reprinted with permission from Ref. [20]. Copyright (2008) Elsevier

varying the concentration of HPG–MA in the aqueous solution as well as by the degree of substitution [21].

7.3 Hyperbranched Polymers as Cell and Tissue Adhesives

Hyperbranched polymers have proved to hold an enormous potential for cell and tissue adhesive. Researchers all around the world have been trying to develop various biomedical agents by utilizing the multiple functionalization properties of hyperbranched polymers. The most significant work has been done by Brooks, Kizhakkedathu, and colleagues. They synthesized HPGs containing multiple choline phosphate (CP) groups. CP has the exact inverse orientation of phosphatidyl choline (PC), which is the end group of the major lipid presented in eukaryotic cell membranes (Fig. 7.7), thereby these functionalized HPGs displayed a strong affinity for biological membranes [22]. Thus, it exhibits a wide range of biomedical application—from tissue sealing to drug delivery. In particular, the researchers took two HPG samples of different molecular weights (23 and 65 kDa) and varied the concentration of CPs attached to them. The multivalent CP-terminated hyperbranched polymers showed strong attachment with human red blood cells, where as the PC-modified polyglycerols bound to the cells very weakly. From fluorescence labeling and tritiation results, it was concluded that the strong interaction between the CP-decorated hyperbranched polymers and the PC-terminated phospholipids was mainly due to the formation of multiple CP–PC heterodimers by electrostatic interactions. Also, a comparison of molecular weights revealed an unexpected result—that for 23 kDa HPG, the binding was stronger which could be due to more stable entropy effect. Further, the membrane-binding capacity of HBs was examined using Chinese hamster ovary cells, where the authors found that the CP-terminated HPGs were rapidly taken up by the cells unlike the PC-modified

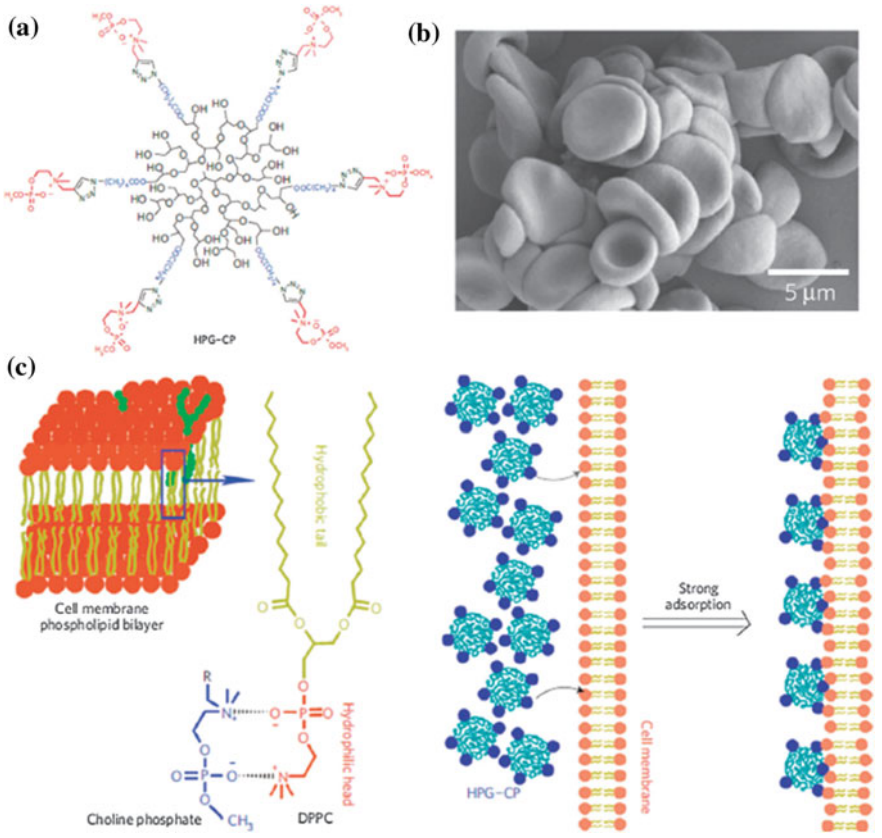


Fig. 7.7 Chemical structure of HPG–CP: multivalent HPG structures (*black*) with CP end groups (*red*) linked by 1,2,3-triazol units (*green*) (**a**) and SEM images (5000) of red blood cells forming aggregates in saline solution as a result of the cell adhesion (**b**) and the mechanism of the biomembrane adhesion interaction (**c**). Reprinted with permission from Ref. [22]. Copyright (2012) Nature (Color figure online)

polymers. Thus, it was finally concluded that these CP-modified HPGs can be also used as drug delivery vehicle.

The characteristics of an ideal tissue adhesive design should be like—the design must be simple and safe, it must have a tunable setting time depending on the application, it must surely be commercial applicable and the use should be inexpensive, painless, and cosmetic. The conventional cyanoacrylate based tissue adhesives have cytotoxicity where as fibrin based adhesives lack in their adhesion properties. Thus, Wang and co-workers designed a simple and scalable hyperbranched poly(b-amino ester) polymer that can be used as strong wet tissue adhesive [23]. They took Dopamine, an amine-derivative of an amino acid abundantly present in mussel adhesive proteins, and copolymerized via Michael Addition reaction, with a trifunctional vinyl monomer, to form a hyperbranched

poly- (dopamine-co-acrylate) (PDA). With fast curing time, low cytotoxicity, and degradable properties, PDA has proved to be a great option as medical sealants and tissue adhesives. The tissue adhesive properties of the PDA polymer was further analyzed by using different curing agents ($FeCl_3$, horseradish peroxidase (HRP)–H_2O_2, fibrinogen and $NaIO_4$) when tested on porcine dermal skin surfaces after curing the adhesive for 15 min, 1 h, and 1 day at room temperature. The test results revealed that Fibrinogen was the best curing agent for achieving a relatively high adhesion strength (37 × 5.6 kPa) within less time (15 min). Another added advantage with these polymer-based adhesives are due to ester backbone, the poly (b-amino ester)-based polymer was able to degrade under normal physiological conditions via simple hydrolysis mechanism. Further when reinforced with nano-sized HA particles (a basic calcium phosphate $Ca_{10}(PO_4)_6(OH)_2$), PDA can find applications such as tunable bone adhesive for sternal closure (Fig. 7.8) [24]. PDA-based nanocomposite exhibits excellent adhesion and mechanical properties

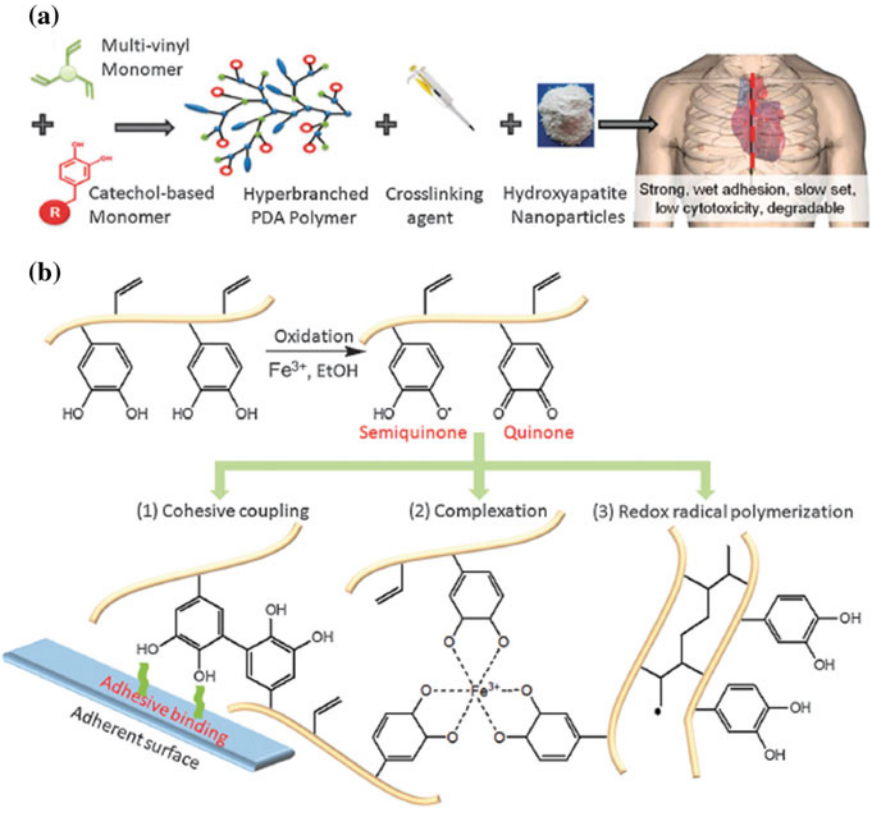


Fig. 7.8 **a** Strategy of using catechol-modified dendritic PDA polymer nanocomposites for sternal closure and **b** the crosslinking mechanism of PDA with Fe^{3+}. Reproduced with permission from Ref. [24]. Copyright (2014) Royal Society of chemistry

which makes it superior to the conventional existing adhesives. Along with that, PDA adhesives have several other advantages such as easy degradation process which is just inversely related to the healing process and also very low cytotoxicity. Therefore, this PDA-based adhesive could be easily commercialized as cell and tissue adhesives.

7.4 Conclusion

Thus it can be concluded that hyperbranched polymers provide a great matrix for cell adhesion and cell growth. With its multibranching sites and wide range of functionality the hyperbranched polymers are regarded as a great component for tissue scaffolds. On the basis of current researches going on with these special architectural polymers, it can be concluded that the field needs to be explored further for better and more specific target. Hyperbranched polymers have triggered the interest around the researchers to develop more biocompatible and efficient tissue component.

References

1. Lin Y-M, Chrzanowski W, Knowles J, Bishop A, Bismarck A (2010) Adv Eng Mater 12: B101–B112
2. Akdemir ZS, Kayaman-Apohan N, Kahraman MV, Kuruca SE, Gungor A, Karadenizli S (2011) J Biomater Sci Polym Ed 22:857–872
3. Puppi D, Dinucci D, Bartoli C, Mota C, Migone C, Dini F, Barsotti G, Carlucci F, Chiellini F (2011) J Bioact Compat Polym 26:478–492
4. Luo Y-L, Zhang C-H, Xu F, Chen Y-S (2012) Polym Adv Technol 23:551–557
5. Kennedy R, Ul Hassan W, Tochwin A, Zhao T, Dong Y, Wang Q, Tai H, Wang W (2014) Polym Chem 5:1838–1842
6. Zhang JG, Krajden OB, Kainthan RK, Kizhakkedathu JN, Constantinescu I, Brooks DE, Gyongyossy-Issa MIC (2008) Bioconjugate Chem 19:1241–1247
7. Mintzer MA, Grinstaff MW (2011) Chem Soc Rev 40:173–190
8. Yang TF, Chin W, Cherng J, Shau M (2004) Biomacromol 5:1926–1932
9. Das B, Chattopadhyay P, Mandal M, Voit B, Karak N (2013) Macromol Biosci 13:126–139
10. Puppi D, Dinucci D, Bartoli C, Mota C, Migone C, Dini F, Barsotti G, Carlucci F, Chiellini F (2011) J Bioact Compat Polym 26:478–492
11. Xu J, Song J (2010) Proc Natl Acad Sci USA 107:7652–7657
12. Lutz J-F, Weichenhan K, Akdemir O, Hoth A (2007) Macromolecules 40:2503–2508
13. Tai H, Howard D, Takae S, Wang W, Vermonden T, Hennink WE, Stayton PS, Hoffman AS, Endruweit A, Alexander C, Howdle SM, Shakesheff KM (2009) Biomacromolecules 10:2895–2903
14. Hassan W, Dong Y, Wang W (2013) Stem Cell Res Ther 4
15. Nikolovski J, Mooney DJ (2000) Biomaterials 21:2025–2032
16. Burdick JA, Anseth KS (2002) Biomaterials 23:4315–4323
17. Nuttelman CR, Tripodi MC, Anseth KS (2004) J Biomed Mater Res, Part A 68A:773–782

18. Knischka R, Lutz PJ, Sunder A, Frey H (2001) Abstr Pap, Jt Conf—Chem Inst Can Am Chem Soc 221, U438
19. Kainthan RK, Janzen J, Levin E, Devine DV, Brooks DE (2006) Biomacromol 7:703–709
20. Kainthan RK, Janzen J, Kizhakkedathu JN, Devine DV, Brooks DE (2008) Biomaterials 29:1693–1704
21. Oudshoorn MHM, Rissmann R, Bouwstra JA, Hennink WE (2006) Biomaterials 27: 5471–5479
22. Yu X, Liu Z, Janzen J, Chafeeva I, Horte S, Chen W, Kainthan RK, Kizhakkedathu JN, Brooks DE (2012) Nat Mater 11:468–476
23. Zhang H, Bre LP, Zhao T, Zheng Y, Newland B, Wang W (2014) Biomaterials 35:711–719
24. Zhang H, Bre LP, Zhao T, Newland B, Da Mark C, Wang W (2014) J Mater Chem B 2: 4067–4071