BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-16-166

Title: Polymer brush: The promising grafting approach to scaffolds for tissue engineering

Article Type: Mini Review

Keywords: Polymer brush; Grafting-to/-from approach; Cell behaviors; Tissue formation; Stem cell engineering

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Running Title:

Keywords: Polymer brush, Grafting-to/-from approach, Cell behaviors, Tissue formation, Stem cell engineering.

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ABSTRACT

Polymer brushes are the soft material units tethered covalently on the surface of scaffolds, which induce the functional and structural modification of a substrate's properties. Due to its facile fabrication, usability of various polymers, ECM (extracellular matrix)-like structural features, and *in vivo* stability, this surface coating approach has attracted special attentions in the fields of stem cell biology, tissue engineering, and regenerative medicine.

Here, we summarized polymer brush-based grafting approaches comparing SAM (self-assembled monolayer)-based coating method in addition to physico-chemical characterization techniques of surfaces such as wettability, stiffness/elasticity, roughness, and chemical composition mainly affecting cell adhesion, differentiation, and proliferation. We also reviewed recent advancements of cell biological applications of polymer brushes focusing more on stem cell differentiation and 3D supports/implants for tissue formation. It is estimated that cell behaviors on polymer brushes in the scale of nanometer length contribute to the systematic understandings of simultaneous effects toward cellular responses at its interface from polymers and scaffolds for promising platform designs.

INTRODUCTION

Polymer brush is a soft material unit with an entangled structure that is covalently tethered on the surface of scaffolds or substrates (1, 2). The polymer brush can assign and tailor diverse structural and functional features of polymers on the scaffold/support surface. Its easy fabrication has further developed and made it applicable in various fields such as electronics, sensors, anti-fouling, catalysis, purification and energy (1, 2).

There have been recent advancements in the coating process of polymer brush for stem cell biology, tissue engineering and regenerative medicine (1, 3) due to the facile attachment of bioactive materials to the polymer brush stimulating the cell to be controlled in specific biological directions, the diversity of scaffold materials for polymer brush coating, and simple fabrication and conjugation process, including utilization of various functional polymers in almost unlimited ways. In addition, the polymer chain length, density and the microstructures of polymer brush can easily be adjusted. It can not only mimic ECM(extracellular matrix)-like structure that induces cell adhesion and growth but it has been reported to be highly stable *in vivo* that it is considered as an optimal candidate in biomedical implants (3).

For biomedical implant to successively develop clinically, biocompatibility of implant materials need to be outstanding, cell adhesion and proliferation to be active so that it can be incorporated onto the host while having good inflammatory resistance and smooth tissue reorganization (4, 5). For this purpose, research in constituting and controlling microenvironments on scaffold surface using the polymer brush for the modulation of stem cell culture and differentiation on its tailored surface is actively under way.

This review summarizes recently reported studies on polymer brush coating for regulation of cell behaviors for bioapplications in tissue engineering and regenerative medicine, as well as cellular responses toward microstructures stemmed from precisely implemented nanometer-sized polymer brush. Recent research in stem cell engineering using polymer brush will be introduced along with studies based on its application in implants and three dimensional (3D) structures with the polymer brush, and finally review the effects of microstructures formed by polymer brush in such a microenvironment and materials that results in complex effects on a cell.

In order to effectively apply the polymer brush in tissue engineering and regenerative medicine, a prior understanding is needed for the procedure of polymer brush fabrication, its physico-chemical features and analyzing processes in characterizing them. This paper describes the properties of the polymer brush and its analysis methodologies focusing on the factors which affect cell behaviors for its application in medical implants.

POLYMER BRUSH AND ITS CHARACTERIZATION

There have been two major approaches in tailoring the physico-chemical properties of an interface by attaching various molecular constituents on the scaffold/substrate, the polymer brush approach and self-assembled monolayer (SAM) approach. Both processes have been intensively applied in cell biological research because these approaches not only give a new function to the surface of the substrates by attaching diverse molecules, but also the resulting tailored interfaces are similar to an ECM (extracellular matrix)'s microstructure, which plays an important role in cellular adhesion, formation and proliferation (4, 6).

SAM, developed by George Whitesides group, describes the molecular assemblies, exquisitely arranged organic molecules that spontaneously form on a substrate's surface in a single layer. Due to self-reorganizing property of these organic elements, it can be coated meticulously. For example, alkanethiols and its assortatively designed patterns via microcontact printing and dip-pen nanolithography on gold thin films of silica glass were widely used in cell research. Regardless of the advantages of SAM, the limited types of useable organic molecules, its unconfirmed stability on other substrates than gold, low *in vivo* stability and its complex fabrication process have limited its application in cell biology and tissue engineering.

The polymer brush approach, which covalently tethers polymer chains on the surface of chemically reactive substrates, have attracted special attention because of its facile fabrication process, compatibility with various scaffold materials such as glass, silicon, gold, silver, titanium, modulation of polymer chain length and density, and additional conjugation of functional molecules. Polymer brush approach can be divided as grafting-to and grafting-from techniques, depending on the difference in its fabrication process. To put it simply, grafting-to technique covalently anchors the polymer chain or polymer unit onto the reactive surface of the scaffold and the grafting-from technique forms a polymer chain or polymer unit via polymerization from polymeric initiating moiety on the scaffold surface. The grafting-to technique forms a looser brush density than the grafting-from technique because of the steric hindrance caused by the previously attached polymers in its coupling process at the interface. According to its fabrication approaches and related parameters, the polymer chain length is adjustable (2). Table 1 summarized and compared various attributes of polymer brush and SAM approaches.

For implants to successfully integrate into the host system, cell adhesion, formation, proliferation, and differentiation has to occur actively on the scaffold-polymer brush interface. The polymer brush on a scaffold which influences such cell behaviors need to be quantitatively analyzed. Some important factors that affect cell behaviors and manipulation include hydrophobicity, roughness, stiffness/elasticity, and chemical composition.

Generally, as the surface hydrophobicity rises, the rate of cell attachment and spreading drops. However, as can be seen in polyethylene glycol (PEG) and poly (sulfobetaine methacrylate) (polySBMA), the hydrophilicity of the polymer brush does not always result in an improved cell adhesion (7, 9, 10). Surface hyrophobicity and hydrophilicity can be measured by the wettability via the increase and decrease in surface tension of liquid drops formed on the substrate surface, which is quantified by the static contact angle measurement. Whereas angle measured from the surface rises in droplets on hydrophobic surfaces, it drops on hydrophilic surfaces because the droplets spread flat (11).

Roughness, a topological property of a surface, plays an important role in cell adhesion, morphogenesis and proliferation especially in implant and tissue formation (7). SEM (scanning electron microscopy) is usually used to observe the overall surface morphology and AFM (atomic force microscopy) is utilized for the quantitative measurement of a more elaborate surface roughness. Roughness via AFM is quantitatively represented by taking the root mean square of the difference between the individual peaks and the average height within specific lines or areas (12, 13).

Rigidity/elasticity, also referred to as stiffness/softness, of the mechanical property of a substrate, is one influential parameter in cell attachment, growth and differentiation (7). Parsons *et al* have shown studies that a cell culture plate where the surface rigidity was modulated caused various types of cultivated cancer cells to alter in cell growth, spreading, proliferation, and migration (8). Surface rigidity/elasticity can be measured by the AFM force mode instead of the image mode. While the cantilever of the AFM tip adheres to the surface, it measures the degree of deflection depending on the surface softness/hardness of a substance.

The chemical composition of the surface needs to be analyzed to determine if polymers or bio-polymers are appropriately attached to the surface. IR and Raman spectrometry is used to identify the substance of the functional groups of chemicals/polymers. X-ray photoelectron spectroscopy (XPS) analyzes the electronic state and element composition of the surface, and thermal gravimetric analysis (TGA) is utilized to detect mass reduction by a rise in temperature, ultimately, monitoring unique phase transition caused by vaporization, sublimation, and adsorption according to its own physico-chemical properties.

In addition, ellipsometry and AFM are used to measure the length of a folded polymer chain (height from bare scaffold), cyclic voltammetry (CV) or surface zeta potential is used to determine the electrochemical properties of a substance, and fluorescence image analysis via conjugation of fluorescent materials is obtained to identify the overall coverage of the target molecules. All these parameters and apparatuses to investigate its surface properties are summarized in Table 2 focusing on the factors which influence cell behaviors.

These tools for analysis enable the micro-architecture and physico-chemical properties of a polymer brush to be characterized precisely, which can directly correlate its features and cell behaviors to use it as a basic coating platform in designing polymer brushes in tissue engineering and regenerative medicine.

POLYMER BRUSH FOR TISSUE ENGEINEERING

The polymer brush has been widely used for protein adsorption, biosensing, anti-fouling, cell culture, and regulation of cell behavior in the field of biomedicine. Cellular responses toward bioactive polymer brush as well as control of its cell behaviors have been especially studied in tissue engineering, and cellular manipulation using thermoresponsive polymer brush has also been under active research.

Currently, studies on polymer brush expand from regulation of stem cell behaviors to 3D support/implant coating for its application in regenerative medicine and clinical biomedicine. And the delicately designed micro-architectures of polymer brush of nanometer-sized chain length and resulting cellular response to both polymer and scaffold material is being systematically investigated. It will lead to collective research on polymer brush for tissue engineering to design the most optimal platform for clinical implant.

Stem cell studies on polymer brush have been directed to regulate stem cell behaviors by using the polymer brush itself or bioactive properties of additionally attached biopolymers (18, 21): Poly[2(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide] (PMEDSAH) polymer brush was shown to maintain the undifferentiated human embryonic stem cell (hESC) in a long-term culture (19), poly(OEGMA-*co*-HEMA) brushes conjugated with vitronectin (VN) peptide was developed as a platform to culture human induced pluripotent stem cell (hiPSC) long-term (17), growth factors attached to poly(acrylic) acid (PAA) brush regulated the differentiation of mouse embryonic stem cell (mESC) (20), thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm) brush controlled fibrinogen adhesion according to temperature for the study of the adhesion of human mesenchymal stem cell (hMSC) (21), and block copolymer (Pluronic F-127: PF127) brush conjugated with antimicrobial peptide and RGD peptide effectively carried out and promoted antibacterial property and cell adhesion/spreading in tissue engineering (22). Meanwhile, beyond studies on the effects of bioactive molecules on stem cells, recent research is advancing in modulation of micro-architectures of the polymer brush as well as its properties (23, 24, 30, 31, 32) and its effects on stem cells caused by its geometrical features (25, 26, 27, 28, 29).

Lahan *et al* (30) showed via a statistical model that reaction time and catalyst ratio of PMEDSAH in its grafting-from polymerization can control the thickness of the polymer brush, and predicted and proved the wettability transition according to a variation in thickness which changed the arrangement and structure of individual polymer. As a result, the prediction and modification of the elaborate microstructure, as have already been reported, proposed an appropriate basis of PMEDSAH polymer brush architecture for the best culture condition and propagation of human embryonic stem cell (hESC) (31). In addition, studies regarding the thickness of the polymer brush, resulting frictional and mechanical properties such as lateral deformation that affect the adhesion and morphogenesis of human mesenchymal stem cells (hMSC) (32), fabrication of diverse nanopatterns with anti-fouling polymer and cell adhesion biopolymer to determine the effects of spreading and differentiation of epidermal stem cells (23), and the topological effects of the surface of a substrate and decoupling topological effects by polymer brush coating on hMSC (24) have been presented.

Huck and Watt *et al* fabricated a round-shaped geometry pattern using cell resistant poly(oligo(ethylene glycol methacrylate)) (POEGMA) polymer and ECM protein, using lithography technique, as well as investigated the way of how geometry effects epidermal stem cell differentiation and the formation of micro-epidermis that mimic normal epidermal tissues (25, 26, 27, 28, 29). These studies allow the prediction of stem cell formation influenced by microstructures and physico-chemical properties of a polymer brush, and contribute to build the platform of polymer brush for the design of actual implants.

The majority of medical implants are of a three-dimensional structure. Research in polymer brush fabrication of 3D scaffold is actively underway for the study of cell adhesion and tissue formation on such 3D support. PCL (ε-polycaprolactone) fiber was used to form 3D microporous scaffolds and to graft with a POEGMA brush. ECM proteins were additionally conjugated gradiently with the assistance of the polymer brush, which subsequently demonstrated the adhesion of hMSC on the 3D support (33). It was reported that a titanium-based bone implant coated with a POEGMA brush was successfully implanted on a rat's leg after attachment of fibronectin fragments, and it promoted the integration of tissue on this coated bone implant (34).

The hydrogel-based support is a 3D architecture/scaffold that is in the limelight because of its formation of artificial ECM network. Its fabrication methodology of bioresponsive brush by conjugating biopolymers on a hydrogel scaffold has been being researched actively. It was reported that the bioactivity of a retinal precursor cell (RPC) was tested on an agarose hydrogel that patternized the protein brush of sonic hedgehog (SHH) and ciliary neurotrophic factor (CNTF) in 3D hydrogel using a two photon irradiation method (35). Patternized RGD in poly(ethylene glycol) (PEG) - diacrylate (PEGDA) 3D hydrogel using two photon absorption lithography affected cell confinement and migration (36). PEG-based hydrogel simultaneously coupled with integrin-mimcked peptide and MMP (matrix metalloproteinase) substrate was fabricated to study cell invasion for tissue regeneration or bone regeneration (37, 38).

Besides stem cell engineering on polymer brush and polymer brush coating for 3D support, it is rising as an important factor to study delicate changes of its thickness and density that lead to different micro-architectures, and the resulting physico-chemical change that affects cell behaviors. The fabrication process for gradient polymer brush grafting was developed to control the chain length and density (43, 44, 45), and it resulted in various degrees of protein adsorption and cell adhesion according to the gradation of polymer brushes (39). Along with polymer chain length, the microstructure of poly[{2-(methacryloyloxy) ethyl} dimethyl-(3-sulfopropyl) ammonium hydroxide] (PMEDSAH) brush changed, and such simulation results showed that well-modulated thickness of polymer brush affected its changes in micro-architecture and transition of physical properties such as wettability which made designing the most optimal polymer brush possible for stem cell culture (30). A simulation of nuclear pore complex (NPC) based on a polymer brush model resulted in different domain structures that was created by various disordered formation of polypeptides placed in the nuclear pore, and it estimated a formation of a unique polymer brush architecture that regulates an open-close nuclear pore (40).

Interestingly, as the polymer chain shortened within a few nanometers, the microstructure and its chemical features also changed, and cells interacted with not only polymer brush, but with scaffolds, the base materials. Santore *et al* sparsely attached cationic polymer brush, poly(L-lysine) (PLL)-PEG graft copolymers, in anti-fouling PEG brush 7~17nm in height, then observed bacteria clinging to the cationic polymer on the surface while it compressed the nanometer-length PEG brush. This result leads to a deep intuition for cellular interaction at its interface on the short length polymer, low density of polymer, formation of flaw, and heterogeneous coating which can be generated during polymer brush fabrication (41).

Baird *et al* observed that RPL (rat basophilic leukemia) mast cells adhered to poly(acrylic acid) (PAA) polymer brush, a cell repellent, when it was formed into square patterns smaller than RBL cells, and cell membrane accumulated on patterned anti-fouling area. However, at 8 nm brush height, regardless of low cell membrane accumulation, there was active cell attachment which shows that there was a strong interaction with silicon, a scaffold substance that has cell affinity (42).

These results show that as polymer chain length shortens its micro-architectures and physico-chemical properties of the polymer brush also change, and simultaneous interaction between cells and the resulting scaffold and polymer will play an important factor for the polymer brush in tissue engineering and regenerative medicine.

CONCLUSIONS AND PERSPECTIVES

Polymer brush has been as the most optimal scaffold surface grafting approach for biomedical implant, regenerative medicine, and tissue engineering. It is supported by current intensive researches using polymer brush in stem cell engineering and polymer brush coating for 3D support in tissue engineering. Not only the types of polymer used in polymer brush, but polymer chain length and brush density, can determine the modulation of polymer brush microstructure and physico-chemical property, and can deduce the most optimal cultivation condition for stem cell engineering. Furthermore, shortening polymer chain length by a few nanometers to induce simultaneous interaction between polymer and scaffold material with cell can predict the effects in cellular response and tissue formation in low density brush, heterogeneous coating, and flaw during grafting. As a result, planning and fabrication of various micro-architecture of polymer brush, precise analysis and modulation of its physico-chemical properties, and systematic studies on its cellular responses will bring about a better understanding of optimal design of the polymer brush for the application of biomedical implant.

ACKNOWLEDGMENTS

We thank Mr. Gyoung Chan Seo for helping us with the figure, and Dr. Soosang Chae for his valuable suggestions for the manuscript. This work is supported by the Hannam University Research Fund and by Business for Cooperative R&D between Industry, Academy, and Research Institute (Grants No, C0395024) funded Korea Small and Medium Business Administration in 2016.

Figure 1.

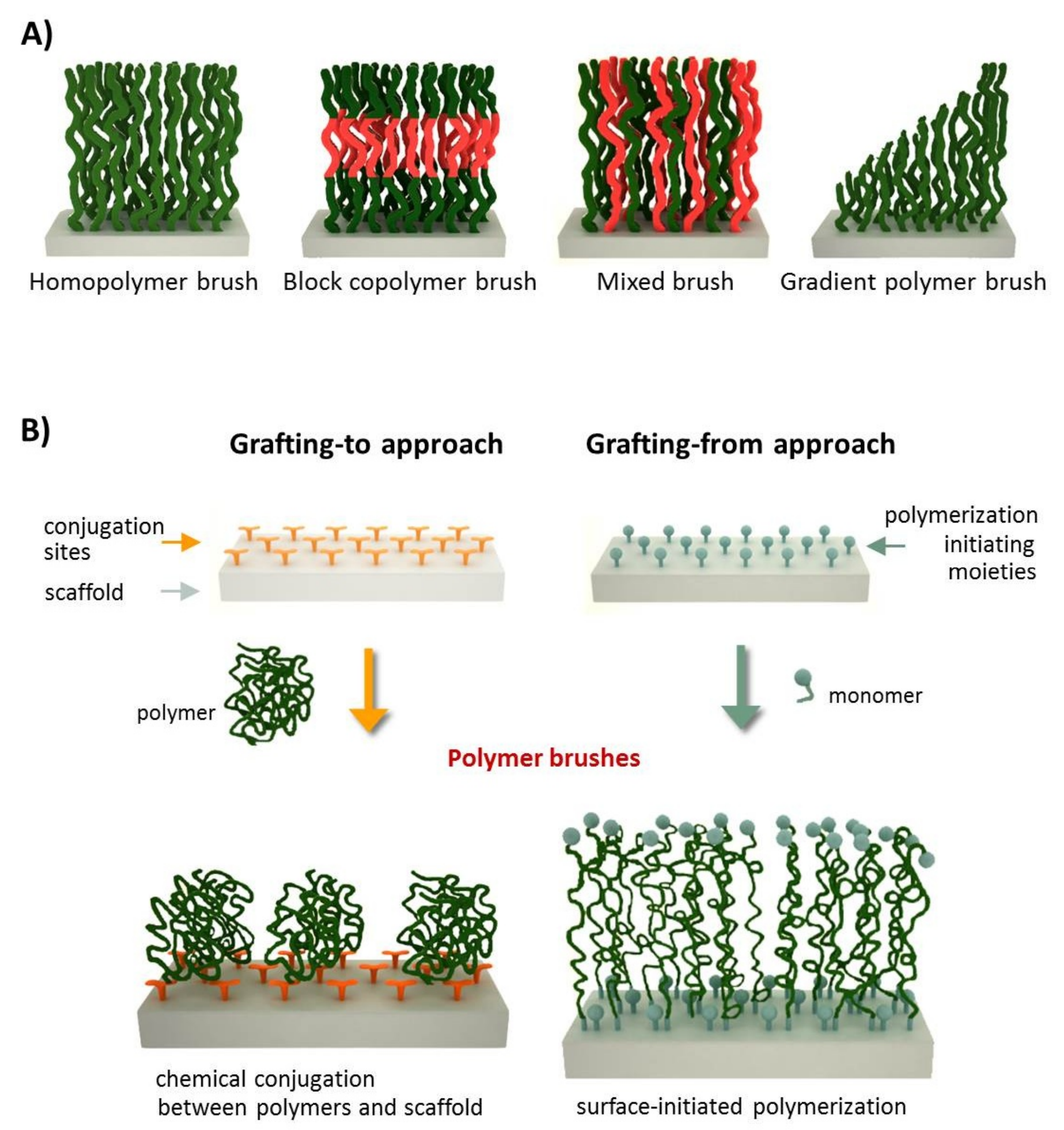
A)
Homopolymer brush
Block copolymer brush
Mixed brush
Gradient polymer brush
B)
Grafting-to approach
Grafting-from approach
conjugation sites
scaffold
polymerization initiating moieties
polymer
monomer
Polymer brushes
chemical conjugation between polymers and scaffold
surface-initiated polymerization

Table 1. A comparison of the physico-chemical properties between polymer brush and SAM (self-assembled monolayer)

	Polymer brush: grafting-to	Polymer brush: grafting-from	SAM (self-assembled monolayer)
grafting molecule	almost all types of polymers		mainly alkanethiol & alkyl silnae
micro-architecture	various and complex polymeric structures		well assembled molecular monolayer
scaffold materials	glass, titanium, gold, silver, silicon, etc		gold thin film, oxide-formed substrate
thickness	high tenability by adjusting polymer chain length		densely packed
coating defects	presence: short polymer chain self-healing of defects: long polymer chain		presence of defects and pinhole
in vivo stability	high stability		low stability
coating density	loosely packed	densely packed	thin: one molecular layer
fabrication approach	various chemical coupling between polymer and surface	various polymerization on the surface	thiol-gold bond & silane linkage

Table 2. A characterization of the physico-chemical properties of polymer brushes on scaffold focusing on the factors affecting cell behaviors.

	wettability	roughness	rigidity / elasticity	chemical composition	height
tools for analysis	contact angle measurement	AFM, SEM	AFM (force mode)	IR & Raman spectrometery, XPS, TGA	ellipsometry, AFM
parameters	angle between surface of a liquid drop and substrate	root mean square of the height of surface contour	the degree of deflection of AFM cantilever at contact point	chemical functional group, element composition, thermal decomposition	height from the surface of scaffold
properties	hydrophobicity & hydrophilicity	topology & geometry	mechanical strength	presence of target polymers on the surface	thickness of polymer brush

FIGURE LEGENDS

Figure 1. (A) Overview of different types of polymer brushes; homopolymer brush, block copolymer brush, mixed brush, gradient polymer brush. (B) fabrication of polymer brushes on scaffolds; Grafting-to approach vs Grafting-from approach. Adapted from ref 2.

Table 1. A comparison of the physico-chemical properties between polymer brush and SAM (self-assembled monolayer)

Table 2. A characterization of the physico-chemical properties of polymer brushes on scaffold focusing on the factors affecting cell behaviors.

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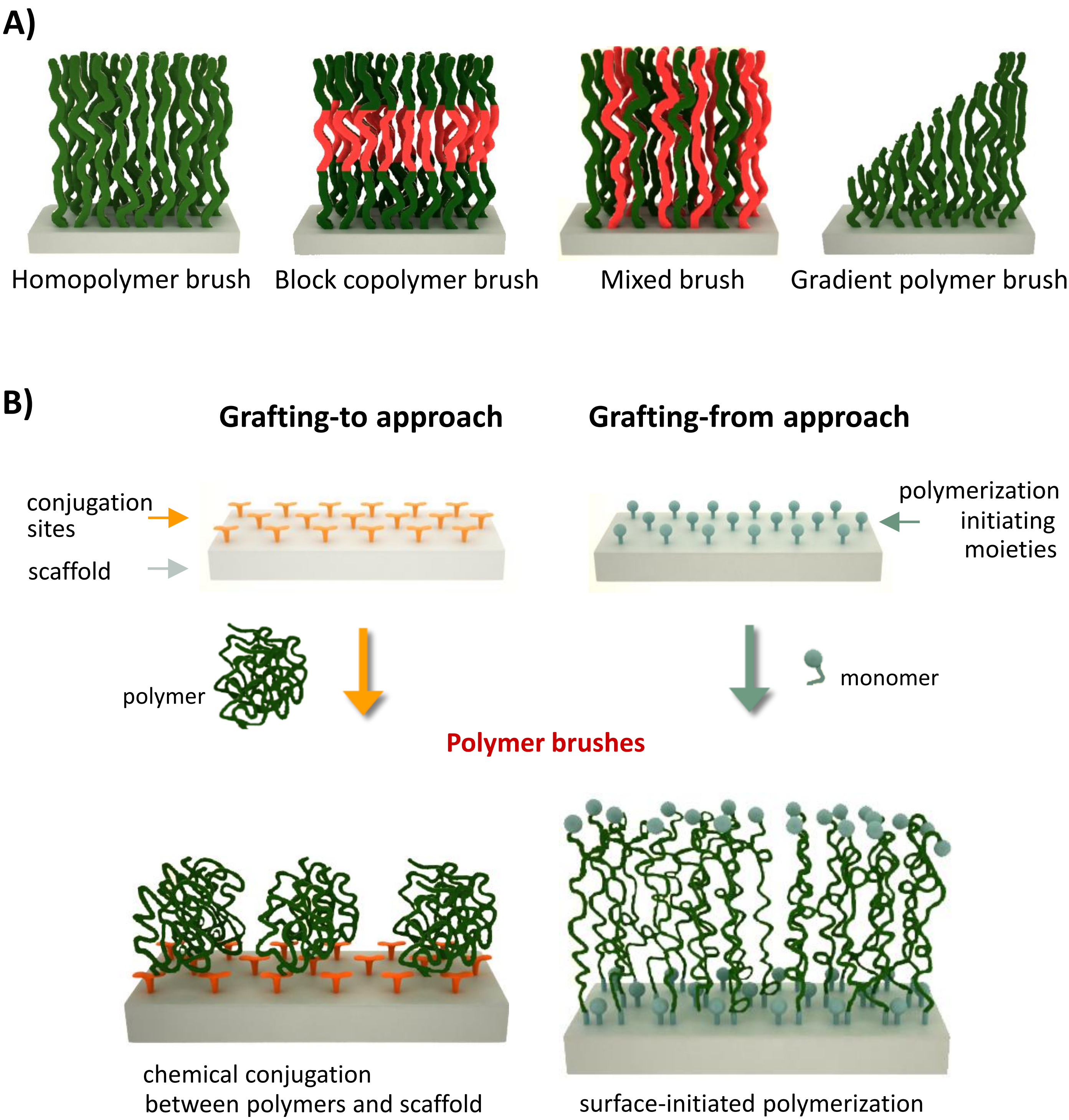
A)
Homopolymer brush
Block copolymer brush
Mixed brush
Gradient polymer brush
B)
Grafting-to approach
Grafting-from approach
conjugation sites
scaffold
polymerization initiating moieties
polymer
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Polymer brushes
chemical conjugation between polymers and scaffold
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Table 1. A comparison of the physico-chemical properties between polymer brush and SAM (self-assembled monolayer)

	Polymer brush		SAM (self-assembled monolayer)
	grafting-to	grafting-from	
grafting molecule	almost all types of polymers		mainly alkanethiol & alkyl silnae
micro-architecture	various and complex polymeric structures		well assembled molecular monolayer
scaffold materials	glass, titanium, gold, silver, silicon, etc		gold thin film, oxide-formed substrate
thickness	high tenability by adjusting polymer chain length		densely packed
coating defects	presence: short polymer chain self-healing of defects: long polymer chain		presence of defects and pinhole
in vivo stability	high stability		low stability
coating density	loosely packed	densely packed	thin: one molecular layer
fabrication approach	various chemical coupling between polymer and surface	various polymerization on the surface	thiol-gold bond & silane linkage

Table 2. A characterization of the physico-chemical properties of polymer brushes on scaffold focusing on the factors affecting cell behaviors.

	wettability	roughness	rigidity / elasticity	chemical composition	height
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parameters	angle between surface of a liquid drop and substrate	root mean square of the height of surface contour	the degree of deflection of AFM cantilever at contact point	chemical functional group, element composition, thermal decomposition	height from the surface of scaffold
properties	hydrophobicity & hydrophilicity	topology & geometry	mechanical strength	presence of target polymers on the surface	thickness of polymer brush