Poly(Vinyl Alcohol) Physical Hydrogels: New Vista on a Long Serving Biomaterial

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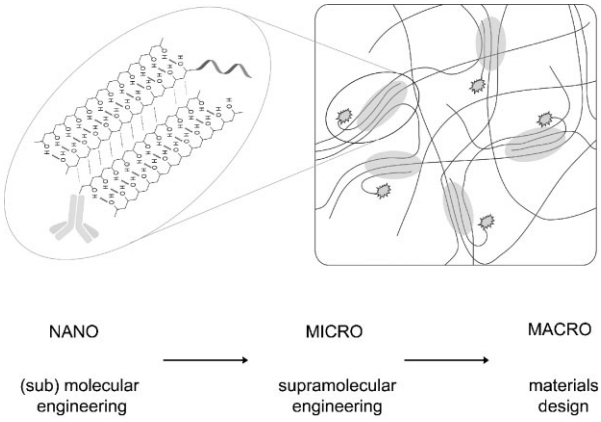
Poly(vinyl alcohol), PVA, and physical hydrogels derived thereof have an excellent safety profile and a successful history of biomedical applications. However, these materials are hardly in the focus of biomedical research, largely due to poor opportunities in nano- and micro-scale design associated with PVA hydrogels in their current form. In this review we aim to demonstrate that with PVA, a (sub)molecular control over polymer chemistry translates into fine-tuned supramolecular association of chains and this, in turn, defines macroscopic properties of the material. This nano- to micro- to macro- translation of control is unique for PVA and can now be accomplished using modern tools of macromolecular design. We believe that this strategy affords functionalized PVA physical hydrogels which meet the demands of modern nanobiotechnology and have a potential to become an indispensable tool in the design of biomaterials.



Introduction

Advances in fundamental and applied biomedical sciences rely on enhanced understanding and a superior level of control over biomaterials, from their synthesis and manufacturing to the design of materials properties, both surface and bulk. Among diverse candidate biomaterials, hydrogels, three dimensional swollen macromolecular networks, offer unique possibilities to engineer materials with properties closely matching human tissue, specifically with regard to mechanical properties, water content, and accessibility to solutes.[1] While chemically crosslinked gels dominate the field, association of polymers through non-covalent linkages, i.e. physical hydrogels, is more friendly toward fragile biological cargo and is therefore highly attractive for biomedical applications. Among these, physical hydrogels based on poly(vinyl alcohol), PVA, stand out due to their superior mechanical properties and biocompatibility.[2,3] These materials have a well-documented history of successful applications in biotechnology and biomedicine, specifically in enzyme and whole cell immobilization, biomass conversion and tissue engineering. These gels are typically obtained through cryogelation, i.e. a benign and non-harmful procedure of freezing–thawing of polymer solutions, a technique which yields robust PVA based materials with excellent mechanical properties, biocompatibility and stability.[2] Regretfully, PVA cryogels largely failed to meet the requirements of nanomedicine (e.g., nanoscale precision in materials design, controlled drug release, and degradation) and as such have not become a common tool for biomedical

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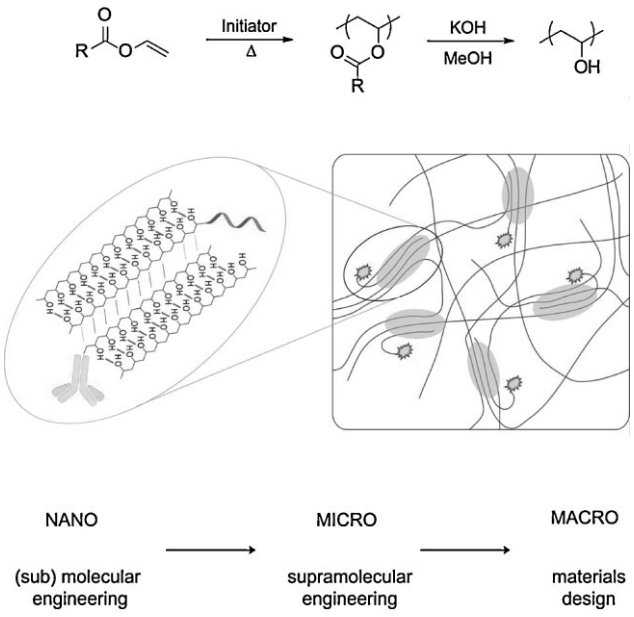

Figure 1. Schematic illustration of a synthetic pathway to obtain PVA (top) and possibilities in materials design associated with this polymer. Modern tools of polymer chemistry allow controlling the stereochemistry of hydroxyl groups (sub-molecular level of control) and polymer molecular weight (macromolecular design), the two factors which define association of PVA chains, i.e. physical polymer gelation (supramolecular engineering), and control the properties of bulk PVA hydrogels (bulk materials design). Together with the recent advances in materials processing and newly developed bioconjugation techniques, this translation of nanoscale engineering to microscale precision and further to macroscale materials design makes PVA a truly unique candidate polymer for diverse biomedical applications.

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engineering. Nevertheless, recent developments in polymer science and nanotechnology, e.g. synthesis of polymers with precise control over molecular weight, novel bioconjugation techniques, controlled supramolecular association of polymers, make it possible to remodel PVA based hydrogels and develop these into stand-alone materials for diverse applications in biomedicine. The aim of this review is to demonstrate that with PVA, like no other polymer, it is possible to exert a sub-molecular and single molecule level of control over polymer chemistry, which can then be translated into superior control over supramolecular association of polymer chains (i.e., gelation) and with this define macroscopic, bulk materials properties, Figure 1. In our opinion, this nano- to micro- to macroscale translation is unique to PVA and offers unprecedented opportunities for biomolecular engineering. We believe that developments in the field will significantly benefit from a multi-angle analysis of tools of control over PVA on molecular and supramolecular level, and this provided an inspiration for this presentation. We do emphasize that this Review by no means provides an exhaustive presentation on biotechnological and biomedical uses of PVA based materials. Specifically, we largely leave out of consideration chemical hydrogels and also only briefly touch on cryogelation-derived materials and refer the readers to earlier reviews on these subjects.[2,4] In the presentation below, we focus on the aspects of monomer, polymer, and material design which contribute to the development of functional physical PVA based hydrogels. In particular, we discuss the available means of control over polymer stereochemistry and outline the existing tools of bioconjugation employed with PVA based materials. We then present the methods to effect polymer gelation and compare the properties of PVA cryogels with those of their counterparts derived by alternative methods. Finally, we briefly discuss the existing and emerging biomedical applications of PVA hydrogels with an emphasis on a broader scope and utility of PVA physical hydrogels made possible by submolecular, molecular, and supramolecular engineering of these materials.

(Sub)molecular Polymer Engineering

General Considerations

Association of PVA polymer chains into a three dimensional network is a spontaneous process and can be accomplished using polymer aqueous solutions, as well as solutions in dimethyl sulfoxide (DMSO), *N*-methyl pyrrolidone (NMP), and mixtures of these solvents. Despite a long history of research and development, the mechanism of PVA gelation remains a topic of active investigation and is far from being perfectly understood. Among the peculiar properties of PVA solutions one can name their ability to undergo a spinodal decomposition,[5] i.e. liquid–liquid phase separation, or form hydrogels through association of polymer chains through a bridging solvent molecule (from their NMP solutions),[6] the latter acting as network junctions; it is also worthy of note that water appears to be only a marginally good solvent for PVA ($A_2 \approx 1.4 \times 10^4$; c.f. for DMSO, $A_2 \approx > 20 \times 10^4$).[7]

Physical gelation of PVA is due to numerous interchain hydrogen bonds between hydroxyl groups and Van der Waals interactions between hydrocarbon polymer backbones. The main tools of control over PVA gelation include polymer concentration and polymer molecular weight, higher concentrations and increasing molecular weights favoring gelation. Remarkably, while this notion has become common knowledge, literature survey reveals that typical studies employ uncharacterized polymer samples and it is also not uncommon to employ PVA with polydispersity indexes around or well over 2.[2] This is largely due to that the development of PVA hydrogels preceded the advent and maturation of controlled radical polymerization techniques (CRP), e.g. reversible addition-fragmentation chain transfer polymerization (RAFT), by several decades. Furthermore, synthesis of PVA differs from that of "conventional" vinylic polymers in that it is obtained by polymerization of a protected "vinyl alcohol", e.g. vinyl acetate, VAc, with a subsequent saponification (ester hydrolysis) of a precursor polymer, poly(vinyl acetate), PVAc, and while polymerization of VAc has been recently explored in detail using the tools of CRP, few studies considered conversion of PVAc into PVA. We therefore strongly emphasize that as such the synthesis of PVA through CRP is still to be performed; fundamental in-depth investigation of PVA gelation, specifically with regard to the influence of polymer macromolecular characteristics on this process and conducted using well defined polymer samples is also yet to be accomplished.

Herrmann and Haehnel (WO Herrmann, W. Haehnel, Ger Patent no. 695048, 1931); appear to be the first to describe the synthesis of PVA from poly(vinyl acetate). Since then and despite a long history, saponification step remains a subject of continued investigation, among other reasons being that PVA is produced globally on a largest scale among other water soluble polymers and that the properties of the polymer are to a great extent dependent on the degree of hydrolysis. Methods to achieve ester hydrolysis include a treatment with strong acid,[8] organic amines,[9] sodium carbonate[10] and, most commonly, methanolic NaOH/KOH. As such, an incompletely hydrolyzed PVAc can be viewed as a copolymer of vinyl acetate and vinyl alcohol and is associated with its own set of physical and chemical properties, including gelation behavior. For this reason and to keep the current presentation within limits, with few exceptions, we describe the properties of PVA with "near complete" (typically >95%) degree of saponification only.

Stereotacticity of PVA

A short range of action of both hydrogen bonding and Van der Waals interactions stipulates that fine-tuning the relative positioning of the interacting chains and hydroxyl groups can have a dramatic effect on the gelation and the properties of the resulting gels. Experimentally, this is achieved through a control in the stereoregularity of the polymer, and in this section we present the means to accomplish this. We specifically aim to demonstrate that the synthesis of stereoregular PVA with associated enhanced gelation capabilities can be run not only in "high end" chemical laboratories but is currently available in virtually any modern chemistry room.

PVA is classified into three groups according to the stereoregularity of the pendant hydroxyl groups; atactic PVA (a-PVA), syndiotactic PVA (s-PVA), and isotactic PVA (i-PVA). Polymer tacticity can be determined by NMR.[11] In ^{13}C NMR, the signals from the methine region can yield a comprehensive analysis of the stereoregularity, extending to pentads. In routine characterization, such an extensive analysis is often unnecessary and typical presentations report the content of syndiotactic dyads (*R*, %). A facile determination of this is available through a ^1H-NMR polymer analysis, typically in DMSO, and integration of the signals corresponding to hydroxyl groups differed in their stereo configuration, namely meso and racemo, Figure 2. The ^1H NMR shows distinct signals from triads (mm, mr, and rr), integrals of which are then used to calculate an average dyad syndiotacticity, $R(\%) = (rr + mr/2)/(rr + mr + mm)$. While commercially available samples of PVA are typically characterized with $R \approx 50$–54%, to the best of our knowledge, the highest experimental value of *R* was that of 74%.[12] However, it is remarkable that a relatively minor increase in *R* from the quoted average value to $R \approx 60\%$ affords polymer samples with dramatically enhanced gelation capabilities and resulting materials properties. Thus, in production of fibrils, the use of s-PVA afforded a higher degree of polymer crystallinity[13] and increased tensile strength;[14] increased syndiotacticity of

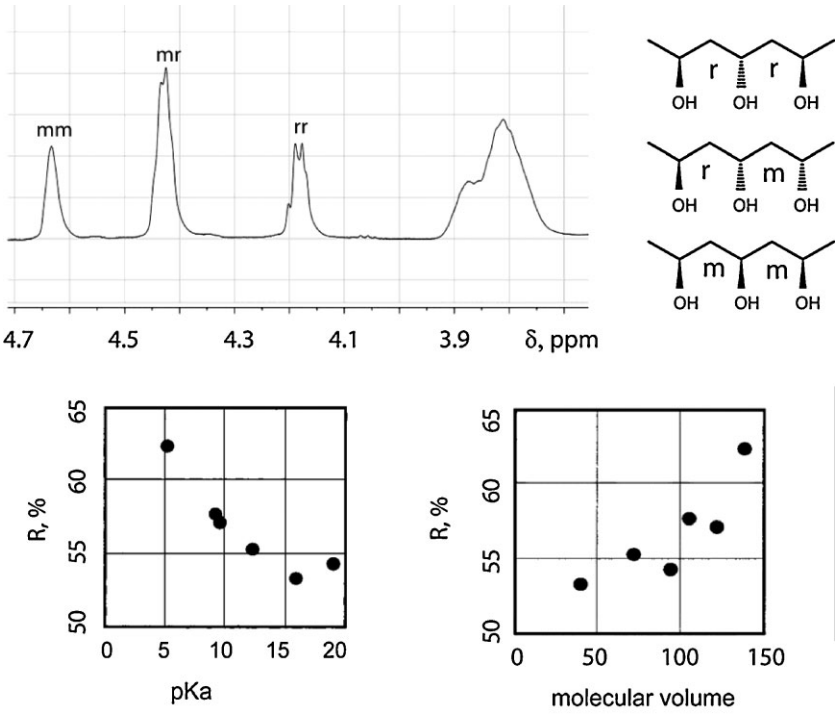

Figure 2. Top: representative ^{1}H NMR spectrum of PVA (in d6-DMSO) depicting chemical shifts for the three possible stereochemical diads (left) and an illustration of diads through relative positions of adjacent hydroxyl groups on a PVA chain (right). Bottom: syndiotacticity of PVA samples synthesized via radical polymerization in fluoroalcohols as a function of the solvent acidicity (left) and molecular volume (right); Reproduced with permission.[31] Copyright 1998, American Chemical Society.

PVA afforded hydrogels with improved mechanical characteristics, e.g. dynamic storage,[15,16] compressive,[17] and elastic moduli[18] as well as markedly increased melting temperature.[19,20] In other words, with PVA, a submolecular level of polymer engineering affords an astonishing enhancement in the degree of polymer association, i.e. allows supramolecular polymer engineering, which ultimately translates into superior control over materials in bulk. This nano- to micro- to macro- translation of control is remarkable and unique to PVA, making this area or research highly attractive for both, fundamental and applied sciences. Below, we discuss the currently available tools of control over PVA stereotacticity, namely the synthesis of polymer precursors using bulky monomers, appropriate solvents, and decreased polymerization temperatures.

Choice of Monomer

The existence of syndiotactic PVA was, to our knowledge, first described by Haas, Emerson and Schuler [9] well before the terms s-PVA and a-PVA have been coined. A sample of PVA obtained from poly(vinyl trifluoroacetate) appeared to be highly ordered in an X-ray diffraction experiment, although the infrared measurements indicated that the material was identical to the "authentic" PVA. Though vinyl trifluoroacetate can indeed be used to make syndiotactic PVA,[18,21] the monomer is expensive and difficult to handle, so more facile approaches have been developed. Steric considerations strongly suggest that a racemo configuration of the adjacent monomer units is more favorable over the meso counterpart, a phenomenon that can be exploited in the synthesis of stereoregular PVA, specifically by choosing bulky monomers. To this end, Nakano et al.[22] explored a range of monomers in the synthesis of PVA, namely VAc, vinyl pivalate (VPi), diphenyl vinyl acetate (DPVAc), and triphenyl vinyl acetate (TPVAc). TPVAc polymerization yielded only oligomers, possibly implying that this monomer is too bulky to polymerize. The use of DPVAc afforded a PVA sample with $R = 65\%$, compared to $R = 62\%$ in the case of PVPi and $R = 53.5\%$ for PVA derived from PVAc. This and many other reports demonstrate that the use of VPi in the synthesis of PVA affords polymer samples with significantly increased R and a corresponding enhancement in the polymer gelation capabilities. However, further increase in the bulkiness of the monomer does not deliver proportionally significant changes in R. This reason, as well as relative commercial availability and price, made VPi a monomer of choice for the synthesis of s-PVA, and it has proven useful on many accounts, both in homopolymerizations[23] and copolymerization with vinyl acetate.[24,25] Lyoo et al.[24] achieved an impressive degree of control over polymer syndiotacticity by copolymerizing VAc and VPi by low temperature polymerization, the latter also favoring increased syndiotacticity. By choosing to do polymerization in THF, a high chain transfer solvent, the authors obtain a low molecular weight polymer with a number average degree of polymerization in the range of 180–360, lower degree of polymerization favoring improved polymer solubility.

While VPi is a monomer of choice when making large scale syndiotactic PVA, saponification of PVPi presents a challenge in the synthesis of s-PVA, and this reaction has proven to be remarkably problematic compared to the hydrolysis of PVAc. Yamamoto et al.[26] report that the success of saponification is highly dependent on the choice of base, the stoichiometric ratio of base, the solvent and the temperature. A major point of concern is the oxidation of the polymer during saponification. To counter this, the authors propose to degas the reaction media, but

problems with degradation persist as the degree of saponification approximates full conversion. The optimal saponification result was achieved in THF and using 6 eq. of KOH at 60 °C for 15 min. The PVA from PVPi can display an unfortunate array of colors due to degradation products. A less than full saponification is also counterproductive, since the remaining ester residues can disrupt the desired physical cross links required for optimal strength of PVA materials.

Other attempts to improve stereotacticity of PVA include a report by Yamada et al.[27] who employed different fluorine containing vinyl esters, and with the di-trifluoromethyl analog of vinyl pivalate, authors achieved a racemo dyad content of 69% by photo initiated polymerization at 0 °C in THF. In another study Fukae et al.[28] investigated an effect of additional polar groups on the side chain and demonstrated a higher *R* value (64%) for a PVA sample derived from poly(vinyl sebacate) as compared to $R = 58\%$ for a PVA obtained through a saponification of poly(vinyl decanoate), the two polymers with structurally similar monomer units and differed by the presence of a polar carboxylic acid functionality in the former. The highest syndiotacticity ever achieved was reported by Murahashi et al.[12] who synthesized poly(vinyl trimethylsilyl ether) through cationic polymerization and obtained a sample of PVA with a racemo content of 74%. The hydrolytic instability of this monomer and the low degree of polymerization make this particular monomer impractical, and the properties of this extremely syndiotactic polymer, besides the melting point, have not been ascertained.

Choice of Solvent

Solvent interactions with both the growing chain and the monomer are also recognized as factors of control over polymer stereotacticity. Thus, Imai et al.[29] reported that a syndiotactic fraction of PVA was markedly affected by the choice of the solvent and decreased in the order phenol > methyl alcohol > ethyl acetate > DMSO, the latter favoring an isotactic propagation. A proposed mechanism for these phenomena is a hydrogen bonding between the monomer and a protic solvent and an associated increase in an apparent bulkiness of the monomer. This effect was further investigated with the use of fluoroalcohols as solvents, a strategy which affords polymers with enhanced stereoregularity for a number of monomers, specifically with pronounced hydrogen bonding acceptor capabilities (vinyl pyrrolidone, acrylamide etc.).[30] For PVA, this was first explored by Yamada et al.: polymerization of VAc in nonafluoro-*tert*-butyl alcohol at 20 °C afforded an astonishing increase in the value of *R* from 53% (polymerization in bulk) to over 62%. The two factors analyzed as contributing to this effect were acidity of the solvent proton and molecular volume of the solvent, defined as the ratio of the solvent molecular weight to its density (Figure 2). The former characteristic is decisive in the affinity of the solvent with the monomer, and the latter contributes to the stereoregularity via steric effects, i.e. an increase in the apparent bulkiness of the monomer, as discussed above. Indeed, an increase in the proton acidity brought about a pronounced increase in *R*; similarly, an increase in the molecular volume of the solvent was followed by an increase in syndiotacticity of the polymer. Interestingly, the described effect of fluoroalcohols was limited to the polymerization of VAc and the discussed trends did not hold true in the case of PVPi[31] and other bulky monomers.[32] In the latter case, the use of nonafluoro-*tert*-butyl alcohol as a solvent favored an enhanced *hetero*-tacticity of the polymers and a significantly decreased content of syndiotactic dyads.[32] Interestingly, while bulkiness of the monomer and that of the solvent exploited together did not result in a synergy of two contributing factors and did not afford enhancement of the polymer stereotacticity, another factor, namely polymerization temperature, was shown to be an effective tool which can be used to further increase the syndiotacticity of PVA. Thus, polymerization of VAc in nonafluoro-*tert*-butyl alcohol at progressively decreasing temperatures afforded an incredible increase in the syndiotacticity reaching 72% for a polymer synthesized at −78 °C.[33]

Taken together, the above analysis reveals that there now exist facile tools of control for both, the molecular weight of PVA and its stereotacticity. Independently, each of these two polymer characteristics was shown to be decisive in gelation of PVA. Concurrent control over *R* and molecular weight therefore presents itself as a tool for enhanced macromolecular design using PA physical gels, and this is a subject of ongoing research in our laboratory.

Molecular Polymer Engineering and Bioconjugation

Despite its excellent biocompatibility and a well-documented behavior *in vivo*, synthetic opportunities associated with PVA are scant as compared to other biocompatible polymers, and as such PVA can hardly be regarded as a polymer in the focus of active research and development of macromolecular drug carrier systems, i.e. polymer therapeutics. Among the primary reasons for this are a limited choice of solvents for PVA (bio)conjugations (water, DMSO, NMP) and a relatively low reactivity of hydroxyl groups and specificity of reactions. Another arresting limitation is that the synthesis of PVA includes a saponification step, typically carried out at harsh conditions. The latter rules out the use of PVA copolymers with e.g., NHS esters, activated disulfides and other activated monomers, the latter being common and convenient tools in polymer-

bioconjugation techniques.[34] As a result, for a polymer with an outstanding biocompatibility,[35] there are solitary examples of PVA based drug carriers. It appears that most of the chemical modifications discussed below, i.e. formation of esters, carbamates, ethers, and acetals, were developed and implemented toward chemical crosslinking of polymer gels; for a broader discussion of these we refer to recent review on the subject.[4] Nevertheless, we strongly believe that PVA based materials have a promising future in polymer therapeutics: we expect that plausible developments and a broader recognition of PVA in bioconjugation, drug delivery, and other biomedical applications will rely on the advances in CRP techniques with associated control over polymer macromolecular characteristics and terminal groups, as discussed in the end of this section.

Chemical Modifications of Polymer Hydroxyl Groups

Direct Coupling

The scope and utility of direct covalent coupling of PVA with candidate drug and/or polymer chains for advanced macromolecular engineering is rather limited and modification of hydroxyls is largely considered to introduce more convenient sites for conjugation, chain extension, and/or substrate immobilization. Examples of the latter include (meth)acrylates,[36–39] thiol groups,[40–43] carboxylic groups,[44–46] halides,[47] and fatty acids.[48,49] In these applications, esters are among the most popular linkages employed,[50–52] and their formation typically relies on PVA reaction with carboxylic acids, acid chlorides,[47] or anhydrides.[44,45] More stable carbamates are typically obtained via reactions of PVA alcohols with isocyanates.[39,42,43,53] Formation of ether linkages was also considered in a number of reports, and this can be achieved through a Williamson synthesis, which is based on the formation of the PVA alcoholate and its subsequent reaction with alkyl halides or sulfones under anhydrous conditions.[54,55] Etherification of the pendant alcohol groups on the PVA with epoxides, specifically using glycidyl acrylate[37] and glycydyltrimethyl ammonium[49] were employed to obtain photosensitive and amphiphilic PVA, respectively. Interaction of hydroxyl groups with aldehydes and ketones in acidic environment, i.e. formation of acetal linkages, is also among popular chemistries for modification of PVA, the most prominent example being a chemical crosslinking of the hydrogels using glutaraldehyde (GA). This method was also used to achieve functionalization of PVA with alkyl chains and obtain hydrophobic PVA derivatives which were able to anchor into the lipid bilayer of the islet (cell) membrane through hydrophobic interactions.[42] Acetalization was used to equip PVA chains with acrylamide groups[56] and conjugated heterocyclic chromophores,[57] as well as photoactive crosslinkable groups and amine functions.[58] In the latter case, the photoactive methacrylate groups allowed hydrogel formation by crosslinking under UV photopolymerization, while the amine functions were used to covalently attach a cell-adhesion peptide RGDS (Arg-Gly-Asp-Ser). A reaction with GA, a divalent aldehyde, can also be used for cargo immobilization into PVA films or surfaces, specifically using the residual free aldehyde groups for further immobilization of drugs and proteins, e.g. enzymes or antigens.[59–61] However, we believe that the use of GA, a highly harmful chemical, for either crosslinking or drug immobilization, is associated with toxicity issues and can be avoided through a molecular engineering of physical PVA gels and alternative conjugation strategies, as discussed below.

Activation with CDI or NPC: Mild and Versatile Strategy

Ossipov et al.[62–64] have extensively studied functionalization of PVA via carbamate linkages using 1,1-carbonyldiimidazole (CDI) as activating agent. The strategy involves a formation of O-(imidazol-1-ylcarbonyl) reactive ester intermediate and a further reaction with nucleophilic species such as primary or secondary amines (Figure 3). In a typical experiment, PVA was treated with an excess of CDI in dry DMSO and then reacted with an appropriate amine overnight at room temperature. Finally, the reaction mixture was treated with aqueous ammonia to hydrolyze unreacted *O*-(imidazol-1-ylcarbonyl)-activated hydroxyls. This versatile two-step approach accommodates introduction of a wide variety of functional groups, such as aminooxy,[64] thiol,[64] aldehyde,[63] hydrazide,[63] azide, and alkyne.[62] Further to hydrogel crosslinking applications, this strategy was implemented in bioconjugation with PVA, specifically for coupling PVA with *N*-(4-hydroxyphenil)retinamide, a synthetic and highly hydrophobic retinoid, used for anti-cancer therapy.[65] The phenol containing drug was added to CDI-activated PVA in NMP, leading to the formation of a hydrolysable carbonate linkage. *In vivo* and *in vitro* studies demonstrated that conjugation with the hydrophilic polymer significantly increased water solubility of the drug and enhanced its antitumor effect against neuroblastoma cells. Other examples of CDI-mediated coupling with PVA include immobilization of lipase onto a hybrid polysiloxane-PVA matrix,[66] a strategy which delivered a total of 95% recovery of the enzyme hydrolytic activity and an increased thermal stability of the protein, and a multi-step synthetic pathway yielding a PVA-paclitaxel bioconjugate with utility in anticancer therapy.[67] Activation of PVA hydroxyl groups can also be performed using 4-nitrophenylchloroformate (NPC), as reported by Arranz and coworkers[68] as an approach for conjugation with 2-amino-2-deoxy-D-glucose (glucosamine). Activation toward efficient bioconjugation can also be performed on a target molecule with

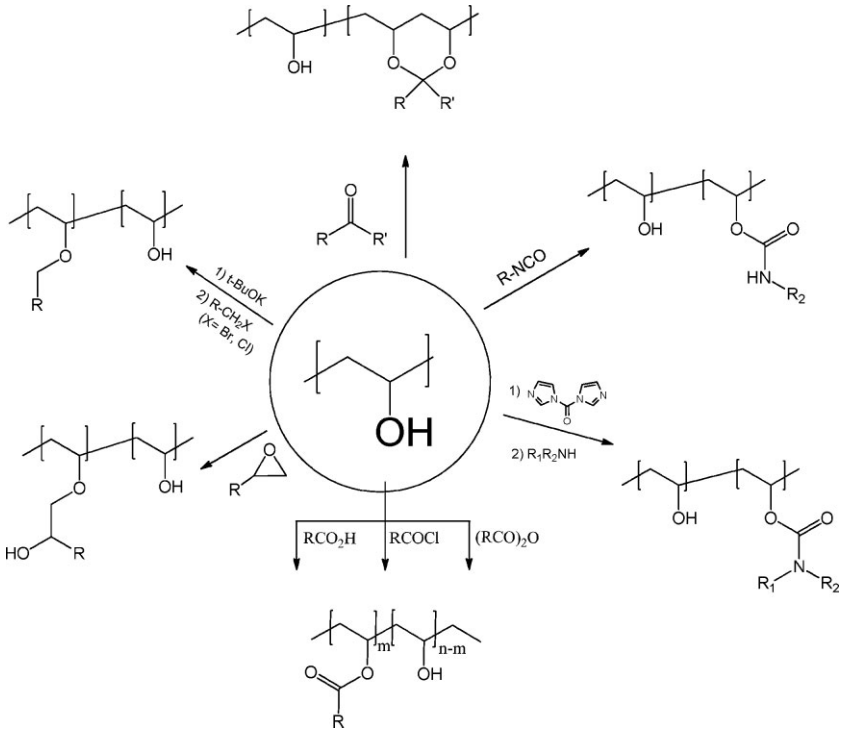

Figure 3. Schematic illustration of synthetic opportunities associated with PVA and conjugation using the polymer hydroxyl groups.

subsequent reaction with pristine PVA through hydroxyl groups, a synthetic scheme pioneered in early 1970s by Schott.[69] Specifically, this strategy was employed for a direct coupling of mono and oligonucleotides covalently bound to PVA by 5′-phosphodiester linkages.[69,70] In another report, chemical modification of PVA by glycosylation was achieved using oxazoline derivatives of triacetylated sugar, e.g. *N*-acetyl-D-glucosamine (GlcNAc) or chitobiose,[71,72] with subsequent deacetylation to yield GlcNAc and chitobiose-PVA conjugates.

Functionalization and (bio)Conjugation Via Terminal Groups

As discussed above, conjugation to PVA through the hydroxyl groups presents viable, albeit scant opportunities to achieve functionalization of polymer chains and/or immobilization of drugs and enzymes on the polymer matrix. Another approach to (bio) conjugation well described in the state of art exploits polymer terminal groups, the latter also being useful in the advanced macromolecular design and the synthesis of block-copolymers, etc. The tools of end group control and modification for PVA are currently scant, yet some prominent examples are described in literature and these will be presented below.

One of the methods to obtain end functionalized PVA exploits sporadic but detectable percentage of 1,2-glycol units resulting from occasional head to head addition steps. Vicinal diols are a convenient site for chain scission using sodium periodate, a reaction resulting in a telechelic PVA-aldehyde (tel-PVA), i.e. a polymer chain bearing aldehyde functionality at each chain terminus.[73–75] This telechelic PVA was used in formation of hydrogels through e.g., reductive amination with chitosan.[76] Telechelic aldehyde PVA can be oxidized to generate a corresponding carboxylic acid telechelic PVA, and the latter was used for cross-linking with the hydroxyl groups of tel-PVA to fabricate a degradable pH-responsive PVA network.[77]

In what appears to be one of the most prominent examples of PVA used in biomedicine, synthesis of thiol-terminated PVA was achieved through a polymerization of vinyl acetate using a radical initiator and thioacetic acid as a chain transfer agent.[78] Saponified polymer, i.e., thiol-terminated PVA, was reacted with maleimide-activated superoxide dismutase (SOD), leading to PVA–SOD conjugates. This conjugation strategy afforded a remarkable increase in protein blood residence half-life (from 5 min to over 3 h) and an almost complete retention of the protein activity. Regretfully, this work attracted little follow up, likely due to poorly established methods of synthesis of PVA with defined terminal functionalities.

Recent advent and maturation of CRP techniques revolutionized the field of polymer chemistry and bioconjugation, bringing the tools of effective control over polymer molecular weight and terminal functionalities into virtually any chemical laboratory. CRP processes rely on a dynamic equilibrium between propagating radicals and dormant polymer chains, and include nitroxide-mediated polymerization (NMP), degenerative-transfer (DT) polymerization with alkyl iodides, cobalt-mediated radical polymerization (CMRP), atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT) involving dithioesters, trithiocarbonates, dithiocarbamates, and macromolecular design via the interchange of xanthates (MADIX). The necessary reagents for most of these techniques are now available commercially and successes of these techniques in bioconjugation are well advertised and reviewed (for RAFT, see ref. [79]). In development of each CRP approach, VAc has been a popular monomer of choice to elucidate the mechanisms and kinetics of polymerization and other fundamental studies, however, few reports focused on the

conversion of PVAc into PVA or preservation of terminal groups through the saponification step.

Redox-initiated radical telomerization of VAc using carbon tetrachloride or chloroform as a telogen was shown to yield trichloromethyl (CCl_3) end-functionalized PVAc.[80–82] The resulting low molecular weight PVAc macroinitiators were successfully used for block copolymerization with styrene[80,82] and methyl methacrylate.[81] Degenerative transfer process using alkyl iodides as transfer agents (leading to a PVAc with controlled molecular weight and relatively low polydispersity) was also reported.[83] However, the iodo-terminated chain ends were unstable and decomposed to aldehyde moieties and subsequently to conjugated structures. Chain end functionalization has also been achieved using cobalt mediated radical polymerization, based on the reversible deactivation of the radicals by the metallic species. After polymerization, all the chains are end-capped by a cobalt(II) acetylacetonate ($Co(acac)_2$) moiety. End-functionalization of PVAc-$Co(acac)_2$ with hydroxyl or epoxy groups could be achieved either by addition of a functionalized non-polymerizable olefin or by displacement of the $Co(acac)_2$ moiety by a functionalized nitroxide.[84] These well-defined PVAc macroinitiators (PVAc-$Co(acac)_2$) were also used to initiate styrene[85] or 1-alkene (1-octene or ethylene)[86] polymerization. However, polymerization of the second block was not always controlled. For this reason, Debuigne et al. converted the cobalt complex attached to chain end of the PVAc macroinitiators into an activated bromide by addition of an α-bromoester or an α-bromoketone containing nitroxide.[87] These bromide-terminated macroinitiators were shown to be effective for the ATRP of styrene, ethyl acrylate, and methyl methacrylate, leading to well defined copolymers. The PVAc-b-PS diblock was in turn converted into an amphiphilic PVA-b-PS by methanolysis. PVAc-$Co(acac)_2$) was also used to synthesize well-defined poly(vinyl acetate)-b-poly(acrylonitrile) (PVAc-b-PAN) block copolymers.[88] The hydrolysis of the ester groups of the PVAc block and the nitrile groups of the PAN sequence of the copolymer provided the double hydrophilic and pH-responsive poly(vinyl alcohol)-b-poly(acrylic acid) block copolymer. ATRP of VAc remains challenging in that the carbon-halogen bonds (C–Br or C–Cl) of the dormant PVAc chains are too strong to be homolytically activated by most of the reported ATRP catalysts, resulting in an extremely low activation constant and therefore a very low ATRP equilibrium. Therefore, almost all the reported highly active copper-based catalysts are inactive in ATRP of VAc. Only recently was reported the first copper-catalyst mediated ATRP of VAc using CuBr/2,2′:6′,2″-terpyridine (tPy) or CuCl/tPy able to yield PVAc end functionalized with bromide or chloride groups.[89]

RAFT and MADIX are among the most investigated CRP techniques as they are probably the most versatile

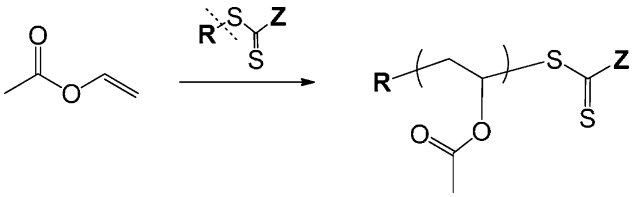

Figure 4. Polymerization via RAFT/MADIX mechanism, specifically using xanthate MADIX reagents, proceeds via "insertion" of monomer units between an R group and a thiocarbonylthio group and yields polymers with terminal functionalities defined by the choice of RAFT/MADIX agent.

processes. Both systems proceed via degenerative transfer processes and differ only in the structure of the compounds employed as chain transfer agents (i.e., RAFT/MADIX agents). RAFT agents are organic compounds possessing a thiocarbonylthio moiety (such as dithioester, trithiocarbonate, dithiocarbamate) while MADIX bear a xanthate group. Success of RAFT and MADIX is based on a combination of the right monomer with the right RAFT agent. A variety of different RAFT/MADIX agents are available, each tailored to a particular group of monomers, and guidelines for selection of RAFT or MADIX agents for various polymerizations are available.[79,90] The activity of the C=S double bond toward radical addition is determined by the substituents R and Z, Figure 4. The *R* group should be a better leaving group than the propagating radical and must efficiently reinitiate polymerization. *Z* is a group chosen to give the transfer agent an appropriate reactivity toward propagating radicals and an appropriate stability to the intermediate radicals. Successful RAFT/MADIX polymerization of VAc has been achieved using dithiocarbamates and xanthates (Z=O–alkyl). A further advantage of RAFT/MADIX is a facile design of end-functional polymers via the judicious selection of RAFT agent structures. The overall process involves insertion of monomer units into the C–S bond of the thiocarbonylthio agent and both the R and ZCS_2 are retained in the synthesized polymer sample. As a result, reactive functional groups can be incorporated either via the R group, the Z group, or indeed both. Hence, an α-functionality can be incorporated onto the polymer chain by carefully designing the R group of a RAFT agent, while the ω-end group of a polymeric chain can be controlled via modification of the Z group or by postmodification of the thiocarbonyl group after polymerization into e.g., a thiol; bi-directional RAFT agents yield telechelic polymers with functional groups on both chain termini. Several functional RAFT agents have already been described in the polymer literature leading to tailor-made PVAc with terminal groups such as thiol,[91] triazole,[92] halogen,[93] azide,[94,95] or carboxylic acid.[96,97] A living character of RAFT/MADIX allows to use these terminal groups for subsequent chain extension, an opportunity realized to synthesize PVAc block

copolymers with PSt,[85,87] NVP,[98] PEG,[99] and PCL.[94] However, while these synthetic opportunities are well available for PVAc, saponification step (e.g., methanolic NaOH/KOH) significantly limits the choice of functionalities in the precursor PVAc which are inherited by PVA. A good case in point, saponification of PVA precursors containing a terminal xanthate group did not lead to the expected thiol-end PVA but led to a complete loss of the CS_2 part and a formation of unsaturated and/or aldehyde structures.[100] Nevertheless, a rational design of PVA terminal functionalities through the choice of appropriate R group in the xanthate is possible, as recently demonstrated by Kostov et al.[8] A xanthate agent with a fluorinated ester-containing R group was employed for the synthesis of PVAc copolymers and creation of fluorinated surfactants. Interestingly, the authors suggest that both the ester-containing R-group and the xanthate moiety were preserved during a conversion of PVAc into PVA conducted in ethanol in the presence of water and sulfuric acid.

Thus, while achievements of RAFT and other tools of CRP in the synthesis of PVA with defined macromolecular characteristics and rationally designed end groups are scant, we strongly believe that synthetic opportunities offered by the tools of modern polymer chemistry are very broad and have a potential to suit a wide variety of biomedical applications. Macromolecular design of PVA with defined molecular weight, stereotacticity, and terminal groups, specifically employing RAFT polymerization technique is the subject of ongoing research in our laboratory and these developments will be discussed in our subsequent publications.

Supramolecular Polymer Engineering

Dissolution of PVA

Understanding and experimental investigation of thermoreversible PVA based physical hydrogels beings with a phenomenon of polymer dissolution. It is typically reported that high dissolution temperatures are required when dissolving PVA with a high degree of hydrolysis due to the strong intra- and intermolecular hydrogen bonds. On the other hand, if the degree of hydrolysis is low, PVA cannot dissolve in water for the reason that there are too many hydrophobic acetate groups present. While this is common knowledge, experimental conditions employed to achieve dissolution of PVA are improvised by each individual research group. Typically the sole criterion used to ascertain completion of the polymer dissolution is an optical clearness of the solution, a feature hardly indicative of a molecular level dissociation of the PVA chains. Furthermore, polymer dissolution from the solid phase competes with solution aging and polymer crystallization, and the latter implies that prolonged incubation at high temperatures may not favor molecular level polymer dissolution. To the best of our knowledge, at present there is no comprehensive study on PVA samples with defined molecular weights to probe the dissolution of PVA.

Cryogenic Gelation

Among the various techniques to effect PVA gelation, a cryogenic route is most well studied and widely employed. This technique was pioneered by Peppas et al.[101] and subsequently attracted significant research attention as an approach to produce robust hydrogels for biomedical applications, specifically tissue engineering. This field is well reviewed and updated by Peppas et al.[102] and Lozynskiy et al.[103] and will be only briefly discussed in this presentation. Cryogels are gel matrices prepared by freezing and subsequently thawing an initially homogeneous polymer solution, Figure 5. The initial freezing occurs at subzero temperatures resulting in formation of ice crystals, the latter acting as porogen material within a polymer matrix, while gradual thawing yields polymer-enriched microphases and association of the polymer chains. The two factors together contribute to a high porosity of the cryogels with typical pore sizes in

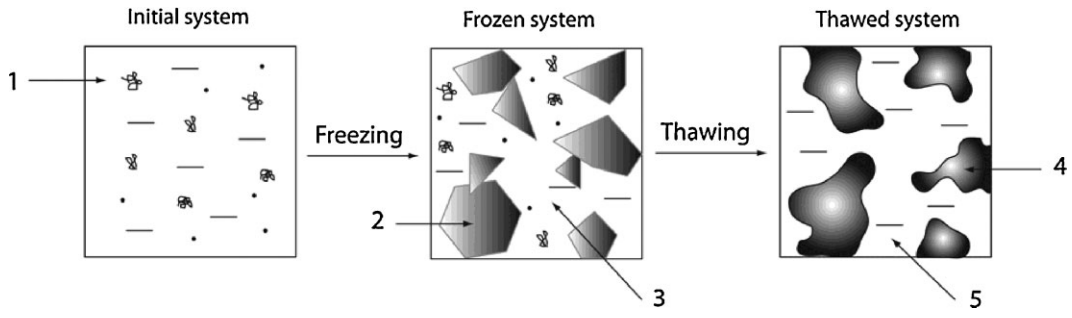

Figure 5. Schematic illustration of a freeze–thaw treatment yielding highly porous PVA physical hydrogels. 1: macromolecules in solution; 2: frozen solvent; 3: liquid microphase; 4: polymer network; 5: macropores. Adapted with permission from ref.[3] Copyright 2003 Elsevier.

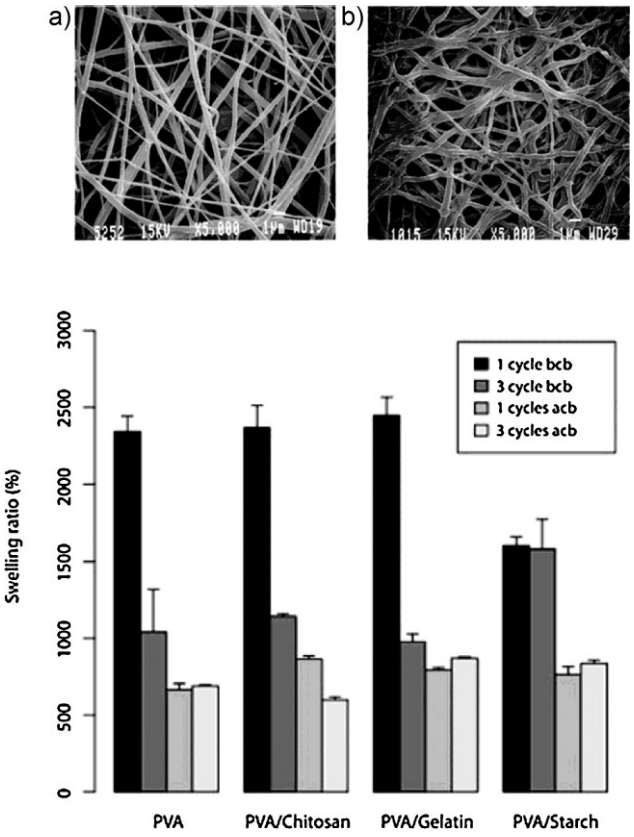

Figure 6. Top: PVA electrospun fibers were successfully stabilized against dissolution in water via a treatment with methanol, the latter acting as an effective water removing agent. Reproduced with permission,[114] Copyright 2003, American Chemical Society. Bottom: a treatment of PVA hydrogels (neat or prepared using PVA blends with chitosan, gelatin and starch) with kosmotropic salts affords samples with decreased swelling (shown in figure) and associated improvement in mechanical properties. Adapted with permission,[123] Copyright 2009, Wiley.

micrometer range and a non-hindered diffusion of solutes and mass transfer. Cryogels prepared as monoliths are elastic, sponge-like gels, and pore sizes of up to a hundred micrometers can be reached.[104]

It is generally accepted that degree of physical crosslinking of PVA chains increases with consecutive freeze thaw cycles.[105] Only ca. 50% of polymer chains are incorporated in a cryogel network after the first freeze thaw cycle and ca. 25% chains still being non-bound after the sixth cycle.[106] Increased polymer concentration and the number of freeze–thaw cycles afforded cryogels with improved compressive elastic modulus toward their applications as artificial lumbar intervertebral disks.[107] Auriemma et al.[108] revealed that for a successful assembly of a macroscopic 3D cryogel, there exists a minimal polymer concentration which suffices interconnection of the polymer-rich microphases into a three dimensional network, and while a 10% polymer solution afforded a robust cryogel sample, 5% solution failed to produce a macroscopic gel under the chosen freeze–thawing conditions. Mechanical properties of the gels can also be controlled by parameters of cryogelation, i.e. thawing rate and holding times,[109,110] and polymer concentration, rather than molecular weight, appeared to be a tool of control over the properties of cryogels.[111] Addition of DMSO into an aqueous polymer solution was shown to aid in controlling both the tensile strength of the cryogels and their porosity.[112] According to the proposed mechanism, DMSO reduces the amount of ice formed and avoids phase separation thus reducing porosity within the cryogel. SEM images of the cryogel prepared by mixed solvents had regularly spaced small pores compared to the irregular spaced and varying sized pores of a regular cryogel. Taken together, PVA macroporous cryogels appear to be well suited for chromatographic bioseparations, cell- and biopolymer immobilization.[3] However, the macroporosity and unrestricted diffusion of smaller molecules significantly limits the use of cryogels as drug carriers, and these gels are further ill-suited for applications that require nano- and microscale control over the surface and/or internal organization of the matrix. For the latter, alternative method of 3D organization of PVA chains are now available, and these will be discussed in detail below.

Non-cryogenic Gelation Techniques

A straightforward method to produce PVA hydrogels was reported by Otsuka et al.[113] The hydrogels were prepared by pouring a PVA solution into a plastic dish and leaving the sample in a closed space at room temperature for 7 d during which the water content decreased by 20 wt.-%. This incubation afforded formation of polymer intra- and intermolecular hydrogen bonds and the resulting hydrogels remained stable when immersed in water over a time period of 60 d indicating the effectiveness of this type of gelation. ATR FTIR and XRD experiments also confirmed the formation of polymer–polymer hydrogen bonds. This is a simple, cost-effective in terms of materials, and benign method of gelation, however, this method is very time consuming when producing larger sized hydrogels.

Another technique widely employed for a controlled removal of water and gelation of PVA solutions is that using water-miscible solvents, such as methanol. Yao et al.[114] employed PVA to produce electrospun fibers which were subsequently stabilized against dissolution in water through an incubation in methanol, Figure 6. The latter step increased the degree of polymer crystallinity and glass transition temperature, the two factors indicative of the formation of physical crosslinks. A 24 h methanol treatment resulted in PVA mats retaining their fibrous appearance after 3 weeks of immersion in water. This study also documents a stabilization of PVA mats by a

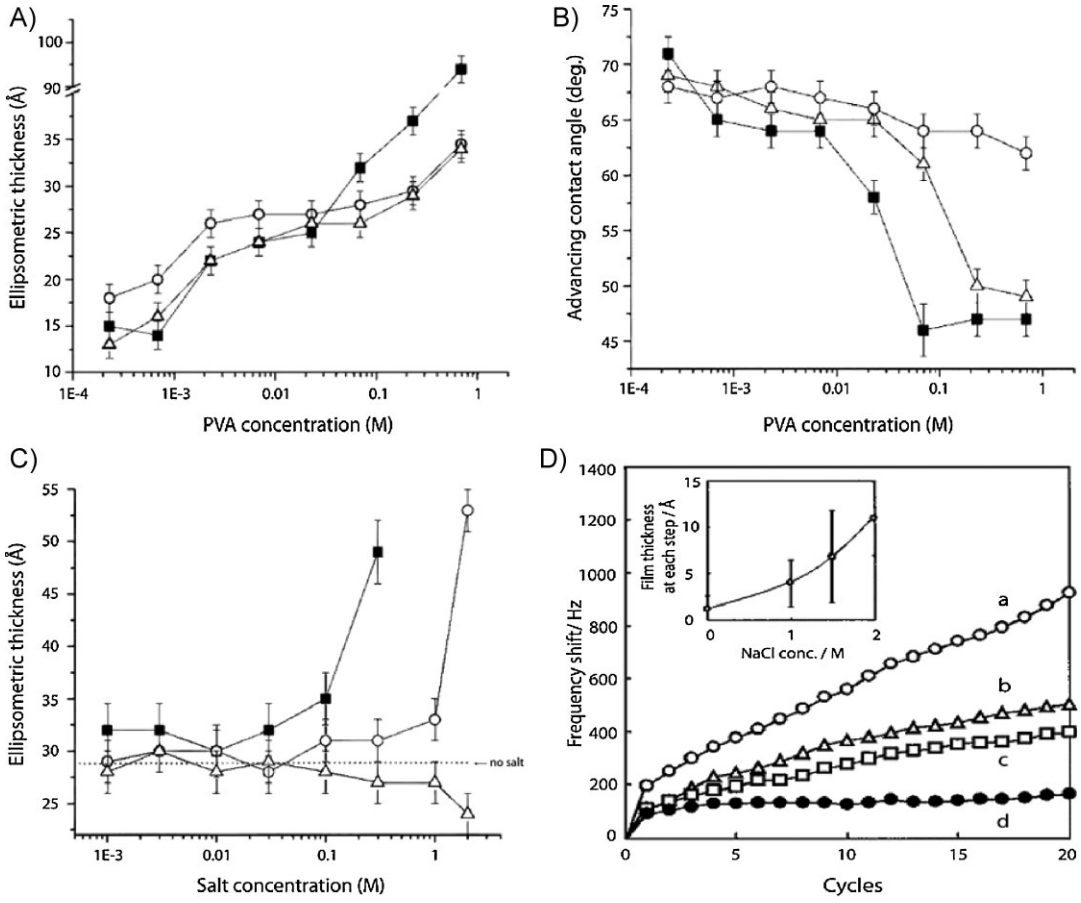

Figure 7. Adsorption of PVA from aqueous solutions onto hydrophobic (A–C) or PVA coated (D) surfaces presents opportunities in surface functionalization for diverse biomedical applications. PVA adsorption onto fluoroalkyl monolayers yields polymer thin films with thickness (A) and advancing contact angle (B) controlled via polymer concentration and molecular weight (○: 14 kDa; △: 89–98 kDa; ●: 124–186 kDa). Polymer adsorption is further facilitated by increased concentrations of kosmotropic salts (C: ○: NaCl; ■: Na_2SO_4; △: NaSCN). Images A–C reprinted with permission,[126] Copyright 2004, American Chemical Society. Adsorption of PVA is also supported by dehydrated PVA surfaces thus allowing for multiple sequential adsorption steps and a build-up of films with controlled thickness, the latter being further controlled by the presence of sodium chloride (D: (a) 2 M; (b) 1.5 M; (c) 1 M; (d) 0 M. The inset is the dependence of the mean film thickness at each step on NaCl concentration). Reprinted with permission,[127] Copyright 1999, American Chemical Society.

treatment with 95% ethanol as well as a 2-propanol/water mixture. A plausible mechanism of polymer gelation involves a solvent-induced dehydration of the chains and a replacement of water–polymer interactions with polymer–polymer linkages. Aliphatic alcohols are non-solvents for PVA and it is therefore also plausible that hydrogelation is preceded by an initial polymer precipitation with subsequent annealing, i.e. rearrangement and crystallization of the polymer chains. In a similar approach, Oviedo et al.[115] prepared physically crosslinked PVA hydrogels by a solvation–desolvation process using acetone. The aqueous PVA solutions were injected with different weight percent of acetone followed by a 72 h drying at 37 °C step. The poorly water-soluble model drug, tolbutamide, was successfully incorporated into the PVA matrix and more drug was incorporated at lower acetone concentration. The PVA polymer chains entangle faster as water is removed at higher acetone concentration causing less drug to be incorporated. It was shown that the drug was released as a function of hydrogel swelling and it is therefore possible to tailor the drug release kinetics by controlling the physical crosslinking in the PVA matrix. This method is fast, inexpensive, and useful when dealing with poor water-soluble drugs.

Demands to biomaterials in use as prosthetic nuclei used for vertebral disks include a match in the mechanical properties of natural tissue as well as integrity of a construct maintained for a prolonged period of time with no shape deformations. Toward this goal, a United States Patent filed by Bao[116] reports on a method where bulky PVA hydrogel samples are prepared via a slow dehydration using a range of water-miscible organic solvents e.g., methanol, ethanol, acetone, 2-propanol, and acetonitrile, as well as ethylene glycol and glycerol. Acetonitrile showed

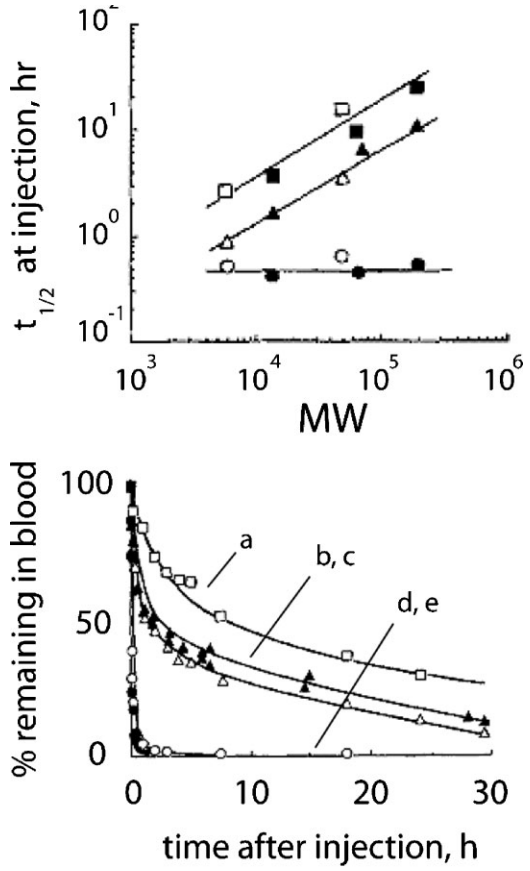

Figure 8. Top: elimination of PVA (solid symbols) and PEG (open symbols) with varied molecular weight from the sites of injection: i.p. (circles), s.c. (triangles), and i.m. (squares). Bottom: decrement patterns of polymer concentration in blood after i.v. injection of PVA and PEG with different molecular weights: PVA 196 kDa (a), PEG 50 kDa, and PVA 68 KDa (b,c), PEG 6 kDa and PVA 14 kDa (d, e). Reproduced with permission,[137] Copyright 1995, Wiley.

the highest dehydration extent followed by 2-propanol, acetone, glycerol, ethanol, and methanol. The degree of shape deformation of the hydrogels upon incubation in these organic solvents also followed the same order for the first four solvents.

A comprehensive study conducted by Choi et al.[117] considered a controlled dehydration of PVA solutions making use of three different methodologies all similar in that an added dehydrating agent effectively draws the water molecules from a PVA surrounding thus favoring polymer gelation. The three agents of choice were: (i) a saturated solution of an inorganic kosmotropic salt, sodium chloride; (ii) a water miscible organic solvent, isopropanol; and (iii) a polymer with a high affinity to water, namely poly(ethylene glycol). Each of these agents added onto a macroscopic PVA sample and incubated for 7–14 d resulted in a pronounced loss of water, the extent of which varied from ca. 10% drawn out by NaCl to ca. 50% extracted by IPA and intermediate values achieved in dehydration by PEG. Subsequent rehydration in saline led to a gain in the sample mass, i.e. hydration of polymer chains to yield samples weighing comparably to the initial, non-dehydrated sample. A repeat in this treatment, i.e. dehydration–rehydration cycling, gave rise to samples with a significantly lower degree of swelling in saline, i.e. dense hydrogel materials. This was already observed after two cycles after which an IPA treated sample exhibited ca. 50% weight loss, and samples of PEG_{600} and PEG_{400} afforded hydrogels with ca. 30 and 20% lower mass as compared to the initial gels. In other words, each of the dehydrating agents afforded non-cryogenic hydrogels with IPA being the most powerful agent, PEG oligomers also being potent gelating agents for PVA solutions, and NaCl being the weakest yet active agent as well. It needs to be pointed out that the initial PVA hydrogels for the dehydration treatment were prepared by a technique termed by the authors as "theta gelation" whereby hydrogels were prepared by casting a hot solution of PVA, 15 wt.-% in water, in the presence of a high concentration of PEG_{400} (28 wt.-%). Subsequent cooling of the solutions led to a phase separation of PVA chains induced by an increased affinity of water to PEG with a resulting porous structure of the gels, not dissimilar to the cryogels. Interestingly, removal of PEG from the hydrogels prior to dehydration treatments (including dehydration by a PEG sample with the same molecular weight) afforded hydrogel materials with significantly decreased equilibrium water content as compared to the gels prepared without intermediate removal of PEG from the "theta" gels. We strongly believe this report was significant for the development of PVA hydrogels and methodologies in the preparation thereof, specifically for applications in tissue engineering.

The use of PEG to effect a controllable dehydration of PVA hydrogels in mild conditions is now well recognized as a powerful approach, specifically for creation of hydrogels for biomedical applications, and this is currently commercialized by GeniaLab, a company which offers ready-to-use PVA solutions, stabilizing solutions, and equipment for reproducible production of hydrogels on industrial scale. These lens-shaped hydrogels, termed LentiKats, were used for successful immobilization of cells and enzymes, as reported in numerous studies.[118–120] Fernandez et al.[121] immobilized inulinase in PVA particles using LentiKat Liquid followed by extrusion directly into PEG_{600} which afforded an instantaneous gelation of PVA. The hydrogels remained stable throughout an extended period of time at elevated temperature (50 °C) indicating efficiency of PEG as a dehydrating and stabilization agent. Worthy of note, PEG-dehydrated hydrogel materials are expected to exhibit a more homogeneous internal structure as compared to the cryogels; nevertheless these gels remained highly permeable to diverse large sized cargo, and immobilization of

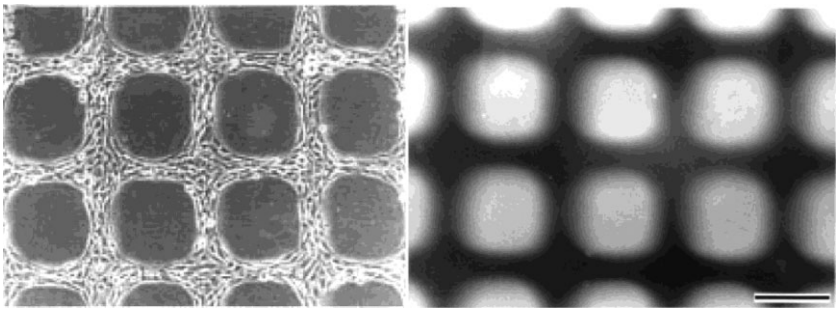

Figure 9. PVA hydrogels were shown to be effective tools of patterning to achieve guided cell adhesion and proliferation (left) as well as surface immobilization of proteins (right). Reproduced with permission,[176] Copyright 1998, American Chemical Society.

protein cargo typically relies on conjugating the enzyme to a carrier polymer.[119,122]

Another approach to gain a better control over hydrogel swelling and mechanical characteristics was reported by Liu et al.[123] who compared PVA hydrogels prepared by conventional freeze–thawing (1–3 cycles) with the samples which underwent a further stabilization through polymer coagulation using aqueous 7.5% KOH and 1 M Na_2SO_4, Figure 6. Equilibrium swelling ratio of the gels was significantly decreased for the gels after three freeze thaw cycles compared to the gels which underwent a single freeze thaw cycle. There was a further significant decrease in the gel swelling upon a treatment in a coagulation bath, the latter treatment also resulting in gel matrices with significantly improved mechanical characteristics. The use of a coagulation bath was also reported by Chuang et al,[124] and taken together these data demonstrate that this treatment yields robust hydrogels which appear to be well suited for diverse biomedical applications.

Crystallization and Gelation Through Adsorption

Another unique property of PVA relates to its propensity to undergo partial crystallization following polymer adsorption onto an underlying surface, specifically perfluorinated hydrophobic surfaces, as explored by Kozlov et al.[125] Polymer adsorption was rather fast and on poly(tetrafluoroethylene-*co*-hexafluoropropylene) surface a PVA film with thicknesses 15 ÷ 35 Å was obtained with incubation time varied from few seconds to 2 d. On self-assembled monolayers based on perfluorinated thiols, deposited polymer film thickness increased with increasing polymer concentration and varied between 15 Å for 0.001 M polymer solution to ca. 32 Å for 1 M counterpart. An increased thickness at higher polymer concentration was explained by adsorption of entangled chains rather than individual macromolecules, a process which also results in coatings with markedly lower advancing contact angles. The latter observation strongly suggests that within thicker films, PVA chains form fewer interpolymer hydrogen bonds and remain hydrogen bonded with water. This, in turn, implies that thicker PVA coatings are less crystalline, an assumption which was verified by ATR IR spectra of the films. In a follow-up study,[126] adsorption of aqueous PVA onto silicon-supported fluoroalkyl monolayers was investigated in detail with regard to the effects of molecular weight, degree of hydrolysis, concentration of kosmotropic salts and temperature on the thickness, wettability and roughness of the adsorbed layers. Supporting the original findings, increased polymer concentration produced polymer coatings with increased thickness, a phenomenon observed on polymer samples with molecular weights 14, 89 ÷ 98 and 124 ÷ 186 kDa, Figure 7A. However, a sharp decrease in advancing surface angle was observed only for the medium and high molecular weight polymer samples and was not pronounced for a 14 kDa sample of PVA (Figure 7B) implying that the film obtained with a low molecular weight polymer exhibits a higher degree of crystallinity. The thickness of the adsorbed layer was effectively controlled by the addition of kosmotropic salts: the latter favor hydrophobic interactions, and in the presence of ca. 0.25 M Na_2SO_4 a single adsorption step gave rise to a 50 Å polymer coating, Figure 7C. A "salting out" potency of kosmotropic salts is further increased at elevated temperatures, and at 80 °C and in the presence of 2 M NaCl, single adsorption step of a 89 ÷ 98 kDa PVA from a 0.023 M solution afforded an astonishing 120 Å thick film. These coatings are expected to be facile tools in modification of hydrophobic surfaces and, with an extensive history of biomedical applications of PVA, can prove useful in the design of intelligent biointerfaces.

A further peculiar PVA adsorption characteristic is that this polymer associates with dehydrated PVA coated surfaces, i.e. exhibits association of polymer chains at a liquid–solid interface and forms a surface adhered hydrogel material. This was investigated in detail by Serizawa et al.[127] who conducted a stepwise (layer-by-layer) adsorption of PVA from its aqueous solutions onto underlying PVA coated surfaces and monitored the amount of adsorbed material by quartz crystal microbalance, Figure 7D. At each adsorption step, polymer deposition was favored by increased polymer molecular weight and concentration and was facilitated by the presence of a kosmotropic salt, sodium chloride. Typical thickness of a thin polymer film deposited at each step was 5 ÷ 20 Å, depending on the deposition conditions and polymer macromolecular characteristics, which implies that with the use of automation

techniques and repetitive adsorption it is possible to obtain PVA coating with desired thicknesses in (sub)micrometer range. Interestingly, thin film build-up was also conducted through an alternate deposition of PVA with poly(*N*-vinylformamide), poly(vinylamine), poly(ethylene oxide), poly(glucosyloxyethyl methacrylate),[127] and even water insoluble poly(methyl methacrylate) when adsorbed from a THF solution.[128] Although the attained thicknesses were lower than for a single polymer PVA films, co-deposition opens further possibilities in surface functionalization and offers diverse functionality. In each of these experiments, surface drying between adsorption steps was found to be a pivotal element in the experiment design: for PVA, repetitive sample immersion into polymer solution without drying did not afford polymer adsorption beyond the initial layer. This observation highlights a high inter-chain affinity observed with PVA, and together with a low fouling character of PVA, this technique provides a unique tool in surface modifications and ligand immobilization.

Nanobiomedicine

The long and successful history of biomedical applications makes it highly promising to revisit and remodel PVA based hydrogels for biomedicine, specifically for applications requiring nanoscale control over materials properties and advanced materials design. This section briefly outlines the existing knowledge regarding the safety of PVA, both for the polymer and hydrogels derived thereof, followed by a presentation of recent advances in materials science and engineering wherein the use of PVA offered significant benefits. We also discuss the cell adhesive properties of PVA and the efforts in cell patterning, and provide an outlook on existing and emerging technologies which may benefit from the use of PVA, particularly when coupled with advances in polymer synthesis and enhanced control over polymer gelation, as discussed above.

Biocompatibility

The available "Final report on the safety assessment of polyvinyl alcohol"[35] presents an exhaustive overview of toxicological studies on PVA polymer and materials derived thereof, starting with a pioneering report by Hueper in 1959.[129] Oral toxicity of PVA was also a subject of a recent comprehensive review by De Merlis and Schoneker, [130] the latter presentation also containing a summary of US FDA approved uses of PVA. Based on a wide literature survey it is concluded that PVA has a very low acute oral toxicity, the polymer is very poorly absorbed from the gastro-intestinal (GI) tract and does not accumulate in the body when administered orally. PVA also exhibits no mutagenic activity in the Ames test.[131] It has to be noted that the discussed data, both favorable and un-favorable, should be quoted with caution, as the original reports often lack the critical information on the polymer synthesis and characterization as well as manufacturing details.

A minimal level of absorption of PVA from GI tract into the blood stream makes this polymer a convenient non-absorbing carrier material for oral administration of drugs. Thus, Veronese et al investigated PVA cryogel materials for *per os* delivery of antibiotics of veterinary interest, namely oxytetracycline hydrochloride and tylosin tartrate.[132] Administration of tylosin in absence of PVA resulted in a fast elimination of the drug with minimal detected level of this therapeutic in organs and tissues. In contrast, when formulated with PVA, significant levels of tylosin were detected in e.g., kidneys and muscles thus revealing a promising character of PVA for these applications. Surprisingly, similar effect was not registered for oxytetracyclin, and in this case the use of PVA carrier afforded no benefit. Regardless the therapeutic agent, significant benefits of PVA formulations were found in that these preparations afforded an excellent maintenance of the drug in a dry state, i.e. within the hydrogel, and also an ease of dosage whereby drug loaded gels are simply added to cattle food.[132] The use of PVA in oral drug formulations was also considered for the uses in humans,[133] specifically for delivery of emedastine difumarate, a fast absorbing anti-allergic agent. Interestingly, while PVA was convincingly shown to have a minimal absorption through a GI track even upon a prolonged administration, the polymer had a non-negligible vaginal absorption, as observed in mice.[134]

The fate of PVA and its elimination from mammalian organisms were considered in several studies. Besheer et al.[135] employed spin labeling of 125 kDa sample of PVA and traced the intraperitoneal (i.p.) administered polymer in rabbit urine. Remarkably, even with such a high molecular weight, elimination of the polymer is reported to proceed via renal excretion and is complete within 40 d. We note however that no analysis of polymer retention in the body or excretion through alternative pathways or analysis of the total mass of polymer secreted was presented; therefore the extent of polymer elimination is not clear. Nevertheless, in an independent study, a renal secretion route was also observed for a PVA sample of 196 kDa.[136] In a comprehensive study by Yamaoka et al,[137] the fate of PVA administered via i.p., subcutaneous (s.c.) or intramascular (i.m.) administration was monitored using radiolabeled polymer samples and analyses of the residual amount of polymer at the site of injection, Figure 8. Polymer elimination from the site of injection was the fastest in the case of i.p administration, whereas i.m. administration afforded the longest in tissue residence time and s.c. administration allowing intermediate kinetics of elimination. Interestingly, elimination of PVA administered i.p. was complete within 2 h and was largely

insensitive of the molecular weight of the polymer (14, 68, and 198 kDa samples tested). In contrast, elimination of higher molecular weight polymer was markedly slower from the site of i.m. and s.c. injections. Analysis of intravenous administration of PVA revealed that a 198 and 68 kDa samples had a blood circulation half-life of ≈10 and 2 h, respectively, whereas low molecular weight sample was eliminated from the blood stream within minutes. A further analysis related to the kinetics of polymer translocation into the blood stream from the i.p., i.m., and s.c. sites of injection. Peak blood concentration was observed at later time points with increased polymer molecular weight and reached 10 h for a 198 kDa sample. Finally, analysis of cellular uptake of PVA by the cells in the peritoneal cavity revealed that most of the polymer was found in the extracellular liquid, i.e. the polymer underwent a minimal cellular internalization. Worthy of note, all of the above observations were similar for PVA and PEG with similar molecular weights (Figure 8), a notion strongly suggesting similar translocation routes for the two synthetic polymers.[137]

The use of PVA microparticles as embolic bodies to minimize blood flow into cancerous tissue was introduced as early as 1975,[138] and later presented an opportunity to examine the recovered PVA based hydrogel from a patient after a long term implantation (over 8 years).[139] 150–250 μm sized PVA particles were inserted into branches of external carotid system and ascending cervical artery in a human subject, and when recovered, the specimen had the same size and shape as the unused particles.[139] Resected particles exhibited a minor calcification, yet no inflammation associated with embolization was found. The reported observations suggest that the particles adhered to the vessel wall, then were covered with thrombus and organized into a collagenous mass.[139] Together with a diminished blood flow to the tumor, the use of embolic bodies and an intra-arterial delivery of anticancer drugs was hypothesized to deliver a benefit of a high and prolonged exposure of the tumor to the chemotherapy agent and a lower systemic concentration of the drug.[140] Despite expectations, when administered i.v. in combination with embolizing microspheres, mitomycin C did not exhibit a decreased systemic toxicity. While these results may be discouraging, we strongly believe that with due optimization this strategy does hold promise for a sustained and localized delivery of anticancer drugs.

While most presentations including the above cited work on embolic bodies suggest an excellent biocompatibility of PVA based materials, other reports in the field demonstrate that a contact of human blood with PVA hydrogels *in vivo* results in an elevated level of platelet microparticles, a reduced life span of platelets, activation of leukocytes, and an elevated plasma levels of complement components.[141,142] These reports strongly suggest that an in-depth, molecular level examination of immunological and toxicological effects of PVA hydrogel materials is highly warranted. Nevertheless, as no positive controls tested in a similar way were employed and presented, it remains hard to gauge the significance and severity of the reported findings. Furthermore, the employed PVA constructs were prepared using a GA-crosslinked chemical hydrogels and it cannot be ruled out that the observed effect is due to GA rather than PVA component. Thus, Downes et al.[143] reported that cytotoxicity of GA-crosslinked chemical gels originates from GA components from within the gel, not residual GA in solution, implying toxicity issues for chemical gels. Similar effect may therefore be responsible for the findings reported by Gemmel et al.[141,142] We emphasize that these findings once again highlight a superior quality of the physical PVA hydrogels over their chemical counterparts for biomedical applications.

An important characteristic of any biomaterial and polymers in particular relates to the aspect of biodegradation. PVA is a typical vinylic polymer with the polymer main chain assembled through carbon–carbon bonds, and is generally regarded as a non-biodegradable polymer. In other words, upon administration (e.g., i.v. or oral), it is highly unlikely to register a noticeable level of main chain scission for this polymer. Surprisingly, several species of bacteria were shown to develop a unique capacity for biodegradation of PVA and to use this polymer as a sole source of carbon. A recent review by Chiellini et al.[144] delivers an exhaustive compilation of data and analysis of literature regarding this subject. The main conclusion made is that PVA, the largest volume water-soluble polymer produced on a scale of several kiloton per year, is subject of biodegradation by bacteria, fungi, and yeasts, provided that a stringent condition of a long term acclimation is met. In other words, bacteria adapt to the use of PVA as a sole source of carbon and effectively assimilate the polymer. However, in an "unprepared" environment biodegradation of PVA remains to be an unlikely process. One of the proven mechanisms of degradation includes the action of *secondary alcohol oxidase* enzyme followed by the action of *beta diketone hydrolase*, these two enzymes acting sequentially. It is interesting to note that biodegradation of PVA, unlike that of e.g. PEG, is an endo-degradation process. Perhaps for this reason another point making PVA different from carbon chain water-soluble polymers and PEG is that there is no apparent restriction to oligomeric species for a successful biodegradation. Together with the nature of enzymes involved and their endocleavage action, their extracellular residence, i.e. biodegradation of PVA without internalization, appears to be another crucial parameter making possible a biodegradation of higher molecular weight PVA samples. Polymer biodegradation proceeds in an aqueous environment and is therefore carried out in aerobic conditions, while biodegradation in soil or composting conditions was much slower or completely

abolished. Interestingly, bacteria were also shown to discriminate between PVA samples by polymer tacticity, as reported by Fukae et al.[145] and an isotactic-rich PVA underwent almost a complete degradation within 6–8 d.

Electrospun Fibers

Electrospinning has recently attracted significant research interest as a technique in biomaterials design and is poised to cater for diverse applications, including biomedicine.[146] This approach allows fabricating polymers into fibers with thickness as thick as several micrometers and as thin as at least 100 nm, and accommodates incorporation of diverse inorganic and organic co-solutes. For biotechnology and biomedicine, specifically production of enzyme-containing fibers, PVA is particularly attractive since electrospinning can be conducted using aqueous polymer solution, i.e. conditions non-denaturing for proteins.[147,148] This subject has been recently reviewed by Greiner and Wendorff[146] presenting also a comprehensive list of references on electrospun fibers prepared using PVA. To mention a few, Tao et al.[149] reported on electrospinning of PVA solutions and showed that the results are under an effective control of molecular weight and polymer concentration and this technique affords beads, beaded fibers, complete fibers, and ribbons of PVA. PVA fibers were also obtained using polymer mixtures with collagen and hydroxyapatite particles[150] as well as carbon nanotubes[151] toward the application of fibers in bone tissue engineering. While pristine PVA hydrogels are pH insensitive, electrospinning of PVA/ferritin mixtures afforded a pH-induced contraction of hydrogels.[152] An important observation in the latter work is that nanofibrous hydrogels exhibited a markedly faster response and equilibrated within ≈100 s upon a change in pH, in stark contrast with microfibrous counterparts equilibration of which required up to 30 min. GA-crosslinked PVA fibers were used to immobilize a range of enzymes, including cellulose,[153] acetylcholinesterase,[154] glucose oxidase,[155] and lipase,[156] with envisioned applications of these systems in e.g., flow-through enzymatic reactors. The above examples demonstrate the scope and utility of electrospun PVA hydrogels, and we strongly believe that a rational design of these materials with the use of polymers with defined macromolecular characteristics and gelation behavior will deliver a significant boost in their attractiveness for biomedicine. Existing examples demonstrate that physical gelation (e.g., treatment with methanol)[114,157] provides adequate stability of the gels and bypasses chemical stabilization, e.g. through the treatment with GA, and the associated potential toxicity issues and loss of functionality for fragile cargo. While the examples of the use of electrospun PVA fibers in drug delivery are few, we believe the existing reports presented below will stimulate further development in the field.

Drug release from electrospun PVA fibers was considered by Zhang et al.[158] and Kenawy et al,[159] the latter demonstrating that methanol and ethanol stabilization of the fibers eliminated the cargo burst release phenomenon and afforded a controlled release of the drug over a period of at least 360 h. Zeng et al.[160] reported on PVA fibers as a platform for protein delivery. The non-stabilized, as-spun fibers were immersed in water and their gradual dissolution afforded a continued release of BSA. To slow down the protein release, PVA fibers were surface functionalized via a chemical vapor deposition polymerization of poly(p-xylylene), an approach which afforded a significant decrease in the protein release kinetics. Functionality of the enzyme after incorporation into PVA fibers was verified using luciferase, and the released protein indeed showed enzymatic activity. Taepaiboon et al.[161] investigated PVA fibers for their release of model non-steroidal anti-inflammatory drugs in a setting mimicking transdermal drug delivery (acetate buffer, pH 5.5, as well as diffusion of the drug through pig skin). Surprisingly, for each particular drug and despite the markedly different surface area, the release of the drugs from fibers and cast films was very similar in kinetics and the extent of drug release closely followed the solubility of the drug in aqueous media. This result is consistent with a mechanism of drug release through a hydrogel swelling and subsequent dissolution of the drug. Gutierrez et al.[162] investigated PVA scaffolds with tailored morphologies for drug delivery and controlled release of ciprofloxacin, an antibiotic drug incorporated into the structure of gels as an insoluble additive which gradually dissolved into the surrounding bulk. While this report does not exploit electrospun fibers, we believe these results can be adapted and implemented with the use of spun fibers as well. Weiss et al.[163–165] investigated a novel PVA fibers-based platform toward the delivery of water insoluble drugs. To this end, the fibers were obtained using PVA solutions containing the drugs solubilized in microemulsions. The non-stabilized PVA fibers dissolved in aqueous solutions and released the incorporated microemulsions with a linear profile, ≈60% of the total payload released over the first 60 min of observations, and a subsequent plateau. This strategy showed promise in delivery of antimicrobial agents with bacteriocidal or growth inhibitory effects observed on different strains.[164]

Cell Adhesion

An important characteristic of a biomaterial which defines its utility in diverse applications relates to the protein and cell adhesion on the interface. As typically found for highly hydrated hydrogels, PVA based materials are recognized as low fouling, i.e. exhibiting a low level of non-specific interaction with solutes and being non-favorable sub-

strates for cell adhesion and proliferation. This notion is well demonstrated by Anseth and coworkers[166] in a report showing that serum proteins exhibit a minimal adsorption onto the surfaces comprised of PVA hydrogels. Fibroblasts were shown to have a minimal adhesion to the pristine gels but readily attached and showed excellent rate of proliferation on fibronectin-functionalized gel surfaces. The latter work made use of GA-crosslinked hydrogels and a covalent attachment of fibronectin to the gel surface. This strategy is also applicable to the physical gels, as demonstrated by Padavan et al.[167] who achieved a graft functionalization of PVA hydrogels with poly(amic acid), a surface modification which made the gels well cell adhesive and amenable to sustain cell proliferation.

A facile and highly diverse non-covalent strategy to render PVA gels cell adhesive relies on an incorporation of polymer additives into a gelating solution, i.e. blending pro-cell adhesive polymers with PVA. As discussed above, gelation of PVA is highly tolerant to the presence of added nanoparticles, nanotubes, and emulsion droplets, and these gels accommodate equally well incorporation of macromolecular additives. The latter include a broad spectrum of synthetic and natural polymers, such as DNA,[168,169] chitosan,[170,123] hyaluronic acid,[171] and other polymers. Chuang et al.[124] studied PVA/chitosan blends toward the adhesiveness of the gels to fibroblasts. The physical gels were obtained using a coagulation bath consisting of saturated aqueous Na_2SO_4 and supplemented with KOH. PVA/chitosan gels sustained a greater level of fibroblast adhesion and proliferation compared to their pristine PVA counterparts, as verified by MTT assays conducted over several days of cell culture. Similarly, Koyano et al.[172] demonstrated PVA/chitosan blended gels with chitosan content below 10 wt.-% did not support adhesion and proliferation of fibroblast cells. In contrast, their counterparts with 30 wt.-% chitosan afforded gel matrices which sustain the attachment and proliferation of fibroblasts comparable to that achieved on collagen coated surfaces. Mathews et al.[173] successfully used PVA/chitosan blends to achieve culturing of bovine aortic endothelial and smooth muscle cells. Liu et al.[123] reported on physical gels of PVA blended with chitosan, starch, or gelatin prepared using a cryogenic technique with an optional post-treatment in a "coagulation bath" containing a 7.5% KOH and 1 M Na_2SO_4 solution. Pristine PVA hydrogels sustained the lowest level of adsorption of collagen, the latter used as a model of biofouling and adsorption of pro-cell adhesion proteins from serum. PVA/gelatin gels adsorbed the highest amount of collagen, PVA/chitosan, and PVA/starch adsorbing intermediate levels of protein, and adhesion and proliferation of endothelial cells also well corresponded to this trend. Noteworthy, pristine PVA gels supported virtually no cell adhesion; in contrast, the three polymer blends sustained cell adhesion and proliferation almost on par with tissue culture polystyrene surfaces. We believe this report currently represents the most comprehensive study into the performance of PVA based materials toward their applications in cell culture. It is also noteworthy that the gels described above are *physical* gels and underwent no chemical crosslinking.

Cascone et al.[171] investigated hydrogels prepared using PVA blends with collagen or hyaluronic acid (HA), specifically toward their utility in the field of controlled release of growth factors (GH). GH-containing collagen/PVA films were prepared by solution casting followed by a GA crosslinking method, and these materials exhibited no growth factor release. In contrast, cryogels did exhibit growth factor release with a 3 d lag phase and a subsequent burst release of the protein. For HA-PVA blends, growth factor release was near linear over the first 3 d of observation after which the profile reached a plateau. An increased amount of collagen or HA in the gels favored GH release, i.e. the gels released a greater amount of the immobilized protein, and HA/PVA gels released a greater amount of GH compared to the collagen counterparts. Overall, this work has explicitly demonstrated that physical gels were superior to their GA-crosslinked counterpart in delivery of growth hormones.[75] We believe that optimization of this system using tools of macromolecular design presented above would prove useful for diverse applications, specifically tissue engineering.

Surface mediated drug delivery, tissue engineering as well as diverse biotechnological applications also benefit from a *guided* cell adhesion and cell patterning. These applications rely on the use of low fouling and cell adhesion resistant materials, a property for which PVA hydrogels are prominent. Indeed, PVA was used as an inert background in patterning using tethered lipid bilayers[174] and was shown to be a convenient material to employ in soft lithography techniques.[175] In what we believe the most prominent example of PVA-assisted patterning to date, Nakayama et al.[176] employed on thiolated PVA which effectively adsorbs onto gold surfaces and undergoes gelation via disulfide bonds, Figure 9. Surface patterning was first achieved using gold, and the introduced polymer was adsorbed only onto gold-coated areas. Bovine endothelial cells then were introduced and exhibited adhesion between the PVA coated areas, not on PVA coated surface. Interestingly, when thiolated PVA was mixed with fluorescent IgG, protein immobilization also followed the patterning and was co-immobilized with PVA, presumable within the gel, i.e. physically trapped within the matrix.

Outlook

In their current form, PVA based physical gels find use in diverse areas of biomedicine and recent examples include preparation for oral delivery of insulin,[177] prevention of

post-surgical tissue adhesion,[178] implantable replacement for the nucleus pulposus,[179] etc. An even wider range of potential applications and modes of utility of PVA based materials were explored but has not progressed to cell culture experiments or advanced biomedical characterization beyond materials science. PVA hydrogels were shown to have a one-way shape memory[180–182] and have a potential utility as molecularly imprinted materials.[183] The polymer was shown to have ice blocking and cryopreserving effect,[184] be amenable for layer-by-layer surface functionalization using a number of co-adsorbing polymers[185] and, recently, graphene[186] and was used in the synthesis of micelle-forming block copolymers with potential utility in drug delivery.[187] A further unique strategy to effect gelation of PVA exploits ultra-high pressurization and the obtained gels showed initial promise in the delivery of genetic material[188,189] and antitrombogenic polymers.[190] We strongly believe that each and every of these applications, existing and emerging, would benefit from a control over molecular weight and stereotacticity of the polymer, means of bioconjugation, as well as diverse techniques to effect gelation, as outlined in this presentation. From another perspective, we envision that some of the existing biomedical uses of PVA may significantly benefit from the outlined developments in hydrogelation. A good case in point is an approach to use PVA solutions as topical matrices for a release of miconazole, an antifungal drug,[191] and the use of PVA in islet encapsulation.[192] We strongly believe that PVA based materials are ready to meet the high standard of biomaterials for micro- and nanomedicine and provide unique opportunities in macromolecular biosciences, and hope that the presented accomplishments and, more importantly, revealed shortcomings and areas of potential development will stimulate research in this highly promising field.

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