Morphological Characterization of Hydrogels


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Abstract
Hydrogels are physically or chemically cross-linked polymer networks that are able to absorb large amounts of water. They can be classified into different categories depending on various parameters including the preparation method, the charge, and the mechanical and structural characteristics. The morphological

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structures are differed from hydrogel compositions to preparation method, fabrication techniques, type of hydrophobic substitutes, etc. This chapter addresses an overview of the morphological characterization of hydrogels and impact of these properties in various potential applications of hydrogels. In a first part, morphological characterizations of hydrogels directly prepared from native materials are described. In a second part, morphological characterizations of hydrogels prepared from different derivatives of native materials by physical as well as chemical cross-linking strategies are introduced. In a third part, morphological characterizations of composite type hydrogels including blending composites, polyelectrolyte complexes, and interpenetrating polymer networks (IPNs) are discussed. In a final part, morphological characterizations of inorganic nanoparticles incorporated hybrid hydrogels are described.

**Keywords**

Superabsorbent hydrogels · Hydrogel’s morphology · Hybrid hydrogel · Cellulose

1 **Introduction**

Hydrogel is an insoluble polymeric substance that shows the ability to swell and preserve substantial amount of water in its three-dimensional network [1]. In a different way, hydrogels are explained as polymeric arrangements those exhibit the capability of swelling in water and holding a considerable portion of water (>20%) in their 3D structure, without dissolving in water [2]. Sometimes, to describe the polymeric cross-linked network, the hydrogels and gels are used interchangeably by the biomaterial scientists. Actually whether they are gels or hydrogels depend on their fluidity in steady-state, where the gels are more dilute cross-linked system than hydrogels [3]. This cross-linking is responsible for their insolvability in water because of the ionic interaction and hydrogen bonding [4], and this degree of cross-linking in hydrogels generally determines their mechanical strength and physical integrity [5].

There are numerous ways to classify hydrogels. Nowadays, classification based on physical properties has drawn more importance due to their extensive uses in diversified fields and exclusive characteristics, such as capacity of diffusion and swelling. Based on their physical properties and mode of applications, hydrogels can be classified into three ways, namely, solid, semisolid, and liquids [6]. Nevertheless, as they are fundamentally made of cross-linking network, based on hydrogels’ cross-linking, they can be classified into two groups: (a) physically cross-linked or self-assembled hydrogel and (b) chemically cross-linked hydrogel [7]. In the category of physically cross-linked or self-assembled hydrogel preparation, various methods have been reported by various researchers, such as freeze-thawing [8], stereo complex formation (dissolving each product in water and mixing the solution) [9], ionic interaction (addition of di- or trivalent counter ions result in hydrogel systems) [10], hydrogen bonding interactions [11], and maturation (heat-induced...
aggregation) [12]. On the other hand, chemically cross-linked hydrogel preparations have been documented numerous testimonies, such as chemical cross-linking [13], chemical grafting [14], radiation grafting [15], radical polymerization [16], condensation reaction [17], enzymatic reaction [18], and high-energy radiation [19].

Due to hydrogels mechanical strength, physical integrity, biocompatibility, degradability, functionality, flexibility, and adaptability, they have achieved extraordinary appreciation in various fields of engineering and technology. Mentionable sites of their applications are tissue engineering [20], therapeutic applications [21], drug delivery [22], cartilage tissue, soft tissue engineering, cell scaffold, regenerative medicine and cartilage repair [23], agricultural and horticultural engineering [24], water purification [25], antimicrobials [26], and bio catalysis [27].

The water sorption ability and swelling kinetics of a hydrogel depend on its porosity. Therefore, increment of porosity in hydrogels is regarded as the most important issue to most researchers. It can be achieved in hydrogels either by physical or chemical techniques. The chemical techniques may encompass phase-separation, foaming, lyophilization, solvent casting, particulate-leaching, etc. [28], while the physical techniques may include laser sintering [29] and laser-enhanced surface modification [30]. To portray a complete picture of surface morphology and topography of numerous hydrogels is the prime target of this chapter. With this aim, scanning electron microscopy (SEM), laser scanning confocal microscopy (LSCM), and atomic force microscopy (AFM) of several types of hydrogels are elucidated comprehensively.

2 Morphological Characterization of Cellulose-Based Hydrogel

Hydrogels, a three-dimensional polymer network able to absorb and release large amount of water without dissolution in a reversible manner, has become a behemoth in research field owing to its versatility in application ranging from agricultural water conservation [31] to cancer drug delivery system [32]. Though most of the hydrogels prepared are from synthetic polymers such as those formed by cross-linking poly (vinyl alcohol) [33], poly (N-isopropylacrylamide) [34], poly (acrylic acid) [35], poly (amido-amine) [36], poly(ethylene glycol) [37], and polyacrylamide [38], in recent times, natural polymers, mainly cellulose-based hydrogels, have attracted major attention due to their biocompatibility, biodegradability, and low toxicity.

Cellulose, the most abundant natural polymer, has become an enticing proposition as the base material to develop hydrogels. Because of its biocompatibility and inexhaustible nature, cellulose and cellulose-based hydrogels have been unequivocally used in wide range of applications. Cellulose, due to the presence of hydroxyl groups in the structure, offers an easier way of functionalization which leads to formation of so many cellulose-based derivatives. Hydrogels prepared from cellulose and cellulose derivatives can be modified to meet the demands of diversified product demand [39].

Morphological properties of cellulose-based hydrogels are analyzed by scanning electron microscope (SEM). SEM reveals the porosity and nature of hydrogel
structure. Moreover, the effect of modification on the size of the pore is divulged by SEM images. SEM sample is generally prepared by first swelling the hydrogels, then freezing in liquid nitrogen, and finally freeze-drying and sputtering with gold prior to the SEM observation [40, 41]. Atomic force microscope (AFM) helps to evaluate the topological attributes of the hydrogels, where AFM discloses the uniformity of surface roughness of the hydrogels [42].

2.1 Native Cellulose-Based Hydrogel

Native cellulose-based hydrogels, owing to the presence of many hydroxyl groups, have been prepared through physical cross-linking. One such native cellulose-based hydrogel was prepared by adding a cross-linking agent in cellulose solution. NaOH-urea was used as the solvent to dissolve cellulose for the preparation of cellulose hydrogel with epichlorohydrin as a cross-linker. This was a “one-step” method which used unsubstituted cellulose [43]. Two different posttreatment methods were compared in morphological characterization. One was heating treatment, and the other one was freezing treatment [43].

From Fig. 1, it is clear that the heating posttreatment creates a macroporous inner structure. Moreover, the pore size decreases with increasing cellulose concentration. On the other hand, hydrogels prepared by freezing treatment display a fiber-like structure, a significant difference from heating treatment. The reason behind this clear difference in structure is probably slow and strong self-association of cellulose chain at lower temperature.

Kentaro et al. reported fabrication of hydrogel from cellulose nanofibers by single alkaline treatment only. There was morphological difference in hydrogels prepared by this method depending on the concentration of alkali used [44].

From Fig. 2, both type of hydrogels exhibited similar network structures with micro- and nanopores when observed at 1000× magnification. But observation at higher magnification revealed morphological differences between the two types of hydrogels. Hydrogels prepared by 9 wt.% NaOH demonstrated a network that was formed by aggregation of individual cellulose nanofibers (Fig. 2b and c) [45], while hydrogels prepared by 15 wt.% NaOH showed a sponge-like network structure due to coalescence of the kinky nanofibers (Fig. 2e–g).

2.2 Cellulose Derivative-Based Hydrogel

Biocompatible cellulose derivatives, such as carboxymethyl cellulose, hydroxyethyl cellulose, methyl cellulose, and cellulose acetoacetate, have become an important competent in hydrogel fabrication. These hydrogels can be formed through physical-linking or chemical cross-linking [46, 47].

A superabsorbent hydrogel was prepared from hydroxyethyl cellulose (HEC), a cellulose derivative, and acrylic acid (AAc) by radiation-initiated cross-linking in aqueous solution [48]. Hydroxyethyl cellulose (HEC), one of the most widely used
cellulose derivatives, is known for its application as a stabilizer, coating agent, or thickener. Recently, it has generated interest in hydrogel synthesis. For modifying gel properties, a second polymer was introduced in the hydrogel system. Acrylic acid
(AAc), a polymer with low cytotoxicity and good swelling properties, was incorpo-
rated into the hydrogel to improve gel properties [49]. HEC of three different molar
masses such as $M_v = 90,000$ named HEC90, $M_v = 720,000$ named HEC720, and
$M_v = 1,300,000$ named HEC1300 were used [48].

SEM images from Fig. 3 show the effect of partial replacement of HEC with AAc.
Highly porous structure with large pores was visible on the surface of HEC90/AAc
gels. Though the surface was smooth in hydrogels with 10% AAc (Fig. 3b), increas-
ing AAc concentration brought heterogeneity on the pore surface, which resulted in
granule like structure. This trend was more highlighted as concentration of AAc was
increased to 30%. This could be attributed to homopolymerization of acrylic acid
which led to less homogeneous gel structure.

On the other hand, HEC1300 gels showed a wide pore size with relatively smaller
pores and homogeneous surface (Fig. 3e and f). The difference in pore size between
current and previous gel could be due to higher crosslink density leading to substantial
lower water uptake before freeze-drying. In HEC1300/AAc gels containing 5% AAc, a
denser structure with smaller pores (Fig. 3g and h) can be located owing to the
improved gelation in presence of monomer. Here, heterogeneity of the surface is
more prominent compared to HEC90/AAc gels. This can be a result of high solution
viscosity of high-molecular-mass HEC. As the mobility of acrylic acid is less impeded
by the increased viscosity than the large polymer chains, homopolymerization
becomes dominant which leads to rough network surface. High AAc content has no
substantial effect on the gel properties, but it does impact homogeneity negatively [48].

Hongchen et al. reported a cellulose derivative-based smart hydrogel by combin-
ing cellulose acetoacetate (CAA) and cystamine dihydrochloride (CYS). This hydro-
gel exhibited good response to both pH and redox triggers [50]. Stimuli-responsive
hydrogels have become a new avenue of research because of their potential appli-
cation in sensing [51], cell culture [52], drug release [53], tissue scaffolding [54],
and 3D printing [55]. Smart responsive hydrogels are capable of changing their
size and shape in response to environmental stimuli such as temperature [56],
enzymes [57], pH [58], light [59], and electric and magnetic
field [60]. The pH/
redox dual-responsive cellulose hydrogels prepared from CAA and CYS combined a
pH responsive dynamic covalent enamine moiety and a redox-sensitive disulfide
moiety [61, 62]. They demonstrated capacity to work in physiological conditions
which opens up possible application as smart sensors and targeted drug release.

SEM images were investigated to test morphological change of the hydrogel
under physiological condition such as in phosphate-buffered saline (PBS) solution
(pH = 7.4).

From Fig. 4, it is clear that the prepared hydrogels (Fig. 4a) have honeycomb-like
macroporous structure which is significant for biomedical application due to its
capacity to facilitate drug loading and release, also offering void space for oxygen
and nutrient transportation and cell proliferation [63]. This structure is consistent
(Fig. 4b) in same pH, but when pH is decreased to 3.5 (Fig. 4c), an irregular
deformed morphology is apparent. This is due to the fact that under acidic condition,
the intermolecular enamine bonds, main protagonist in determining gel stability,
were hydrolyzed to amine and acetoacetyl groups.
Fig. 3  SEM images of HEC90/AAc hydrogel with 5% (a), 10% (b), 20% (c), and 30% (d) AAc, pure HEC1300 (e and f) and HEC1300/AAc hydrogel with 5% AAc (g and h). Magnification: ×100 (a–e, g) and ×500 (f, h). (Reprinted with permission from [48] Copyright © 2017, Elsevier)
Jiaojiao et al. fabricated a stimuli responsive hydrogel, prepared from hydroxyethyl cellulose and lignosulfonate-graft-poly (acrylic acid), having semi-interpenetrating network (semi-IPN) structure [64]. Lignin, the second most abundant natural polymer after cellulose, is generated in large amount during pulping process all over the world [65–67]. Lignosulfonate (LS), generally treated as a waste product in pulping industry, offers sufficient reactant functional groups to make it an attractive component in different applications [68–70]. Hydroxyethyl cellulose (HEC), a hydrogen-bond acceptor, was combined with acrylic acid (AAc), a hydrogen bond donor, and lignosulfonate in HEC solution by in situ polymerization. These hydrogels exhibited good mechanical properties and stimuli-responsive swelling properties which can open door for many potential new applications [64].

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From Fig. 5, hydrogel containing no lignosulfonate shows less homogenous structure with nonporous region (Fig. 5a). Compared to this, hydrogel with 7% lignosulfonate content exhibits a highly porous honeycomb-type structure with regular pores having 11.2 micrometer average diameter. These pores form capillary channels, which leads to high accessibility of water to the amorphous region of the hydrogel giving it as high as 85% water absorption capacity [71]. So, the morphological changes on the hydrogel have significant effect on its application as superabsorbent [64].
2.3 Cellulose-Biopolymer Composite Hydrogel

Cellulose and different biopolymers, such as gelatin, alginate, starch, and hyaluronate, have been blended to fabricate hydrogels with excellent properties applicable in various applications. Treesuppharat et al. successfully combined bacterial cellulose with gelatin to prepare hydrogel composite material useful in drug delivery systems [42]. Gelatin, owing to its attractive properties like high water absorption, non-toxicity, and biodegradability, has become a notable contender in biopolymer-based hydrogels fabrication intended to be used in drug delivery systems [72–74]. Bacterial cellulose, one of the most effective reinforcement materials, has structure similar to plant-based cellulose but offers added advantages such as high specific surface area, high degree of polymerization, and high crystallinity. Presence of bacterial cellulose in cellulose-gelatin hydrogel imparts tensile strength and dimensional stability when put under externally applied forces [75, 76].

From Fig. 6, pure gelatin and bacterial cellulose-gelatin hydrogel both exhibit porous structure due to removal of solvent. The pores have spherical shape in general and no specific size. Importantly, the size of the pores decreased as the amount of gelation in the hydrogel was decreased. So morphological properties was affected by the ratio of gelatin and bacterial cellulose [42].

From Fig. 7, topology of gelatin and bacterial cellulose-gelatin hydrogel was analyzed, and it revealed uniformity of surface roughness. It is clear that bacterial cellulose has higher degree of roughness. But in hydrogels, bacterial cellulose entered the cavities of gelatin and exhibited increased smoothness. Consequently, both SEM and AFM images disclosed the morphological change with decreasing gelatin percentage in hydrogel [42].

Linseed gum-cellulose hydrogels were developed by mixing cellulose and linseed gum in NaOH/urea solvent and epichlorohydrin was used as cross-linking agent [31]. This hydrogel was prepared with water conservation as the intended application, as linseed gum possessed good water retention capability owing to its
Fig. 6  SEM images of bacterial cellulose and gelatin hydrogels (a) pure gelatin and hydrogels with gelatin-to-bacterial cellulose ratios of (b) 25:1, (c) 50:1, (d) 100:1, (e) 200:1, (f) 300:1, (g) 400:1. (Reprinted with permission from [42] Copyright © 2017, Elsevier)
high swelling ratio and viscosity. But as linseed gum exhibits weak gel properties, it was combined with cellulose to impart toughness. Moreover, chlorohydrin was added to bring its superabsorbent property to the fabricated hydrogel [77]. The linseed gum-cellulose composite solutions were prepared by mixing linseed gum (LG) and cellulose (C) solutions with ratio of 4:6, 3:7, 2:8, 1:9, and 0:10 (ratio of solid contents of linseed gum and cellulose) by weight, respectively. The composite hydrogels were named as G1 (LG-C ratio 4:6), G2 (LG-C ratio 3:7), G3 (LG-C ratio 2:8), G4 (LG-C ratio 1:9), and G5 (LG-C ratio 0:10) according to the ratio of linseed gum to cellulose.

From Fig. 8, it is clear that the hydrogels had three-dimensional macroporous inner structures. This porous structure facilitates the permeation of water, which allowed water to penetrate the inner structure. As a result of absorption of water, the space in
the networks of hydrogels was increased. This was due to electrostatic repulsion from the anionic groups in linseed gum [78]. The average size of pore increased from 18 to 28 micrometer with increasing gum content in hydrogel, from G4 to G2. This in turn, resulted in higher absorption of water which led to a loose structure. Conversely, the average pore size of G3 and G4 was smaller than G5, which could be attributed to failure of linseed gum to support the hydrogel structure in the same way cellulose does. Consequently, linseed gum contributed to controlling the pore size; on the other hand, cellulose played a role as backbone to strengthen the porous structure [78].

2.4 Cellulose-Inorganic Nanoparticle Hybrid Hydrogel

Cellulose-inorganic nanoparticle hybrid hydrogels have garnered interest due to potential applications in optical, magnetic, electronic, and biomedical fields [79]. Monireh et al. have conducted a study on superabsorbent hydrogel based on carboxymethyl cellulose (CMC) and graphene oxide nanoparticles. This hybrid hydrogel was prepared, with controlled drug delivery as intended application, by physically cross-linking cellulose and graphene oxide nanoparticles with FeCl₃.6H₂O. In contrast to pure polymer hydrogels, nanoparticle-incorporated hydrogels exhibited better results owing to their stronger physical, chemical, and biological properties [80, 81]. Graphene oxide (GO), due to biocompatibility, low toxicity, and amphiphilic nature owing to the functional groups such as hydroxyl, epoxide, and carboxyl on the surface, is an attractive candidate in drug delivery
system, which also happens to greatly enhance the mechanical properties of CMC [82–84]. CMC, a biodegradable polymer with multiple carboxyl groups, exhibits good coordination with metal and thus forms excellent hybrid hydrogels [85].

From Fig. 9, it is clear that GO nanosheets are visible (Fig. 9a and b). Pure CMC hydrogel exhibits rough structure and visible wrinkles on the surface (Fig. 9c), while CMC/GO hydrogel containing GO has smooth surface due to cross-linking effect of intercalated GO sheets (Fig. 9d–f). These sheets make strong H-bonding interaction with functional group of CMC, thus increasing smoothness of the surface [32].

Another hybrid hydrogel was prepared from hydroxypropyl cellulose (HPC), a cellulose derivative, and inorganic nanoparticle such as molybdenum disulfide (MoS2) with intended application as dye absorbent [86]. Hydroxypropyl cellulose, an important cellulose derivative due to its high water solubility, has been used to remove dyes from aqueous solution [87]. But its relatively low absorption capacity has limited its application as absorbent [88, 89]. To counteract this limitation, incorporation of nanoparticle with large surface area and multiple functional group is often deployed [90, 91]. Moreover, addition of nanoparticle to the hydrogel system improves mechanical properties of cellulose-based hydrogels. Molybdenum disulfide (MoS2) has been used in the fabrication of hydrogels due to its two-dimensional structure, which is expected to improve

Fig. 9 SEM images of GO (a and b), CMC hydrogel (c) and hybrid hydrogel of CMC/GO with different GO content (d-5%, e-10%, and f-15%). (Reprinted with permission from [32] Copyright © 2017, Elsevier)
absorption capacity of the hydrogels. In addition to this, MoS\textsubscript{2} has been widely used as photocatalysts, which catalyzes the degradation of organic dyes facilitating its removal from aqueous system [92].

From Fig. 10, SEM image of HPC hydrogel shows a loose 3D network structure of porous nature. This is due to phase-separation upon increasing reaction temperature to 50 °C. While MoS\textsubscript{2}/HPC hydrogel shows no distinct MoS\textsubscript{2}/HPC nanosheets, which indicates a uniform distribution of MoS\textsubscript{2}/HPC nanosheets in HPC [93]. Moreover, a smaller pore size than that of the previous hydrogel was observed as a result of addition of MoS\textsubscript{2}/HPC, which might have reduced the expansion of the gel matrix leading to decrease in pore size [41].

3 Morphological Characterization of Chitosan-Based Hydrogels

Chitosan is the deacetylated product of chitin (\text{N-acetyl-d-glucosamine}) which is the second most abundant natural biopolymers after cellulose. Recently chitosan and chitosan-based hydrogels have gained considerable attention for their unique properties like stability, biocompatibility, stimuli sensitivity, biodegradability, bacteriostatic effects, and mechanical strength to be used in numerous applications like in drug delivery, protein release, tissue engineering, dye removal, wastewater treatment, etc. [94–96].

Chitosan and chitosan-based hydrogels can be prepared by physical, chemical, or radiation-induced cross-linking, and the physical characteristics like pore size, distribution of pores, and wall thickness vary from one method to another. To tune these parameters for intended application, morphological characterization is essential. Morphological characterization reveals the shape, size, porosity, and size distribution of pores of the hydrogel which must be known to control or tailor these parameters for the desired application [97].

Morphological analysis of hydrogels is quite difficult due to the delicate nature of the system and chance of collapse of pore structure during dehydration. However
a number of techniques are available for microstructural analysis of hydrogels like scanning electron microscopy (SEM), laser scanning confocal microscopy (LSCM), confocal laser scanning fluorescence microscope (CLSM), fluorescence microscope (FM), etc., but the contrast and resolution of the microscope system are very important; lack of which can lead to poor images [98].

3.1 Scanning Electron Microscopy of Chitosan-Based Hydrogels

For morphological characterization of hydrogels by SEM, special sample preparations are required. To observe the surface morphology, after gelation, hydrogel samples are quickly frozen in refrigerator or in liquid nitrogen and further lyophilized with a freeze dryer system under vacuum at $-50 \degree C$ to $-70 \degree C$ for at least 48 h until all of the water is sublimed [99]. The sample is then mounted on a metal stub usually made of aluminum with conductive tape and is coated with metal for conductance under vacuum by a sputter. Gold is used to coat the freeze-dried sample widely, but gold-palladium or platinum are also used [96, 100]. To observe the interior morphology, i.e., cross-sectional view of hydrogels, the freeze-dried hydrogels are usually fractured and then sputter-coated with metal [99].

When chitosan is chemically cross-linked to prepare hydrogel, the degree of cross-linking can affect the bulk and surface morphology of freeze-dried hydrogels. In case of hydrogel of chitosan prepared by chemically cross-linking with glutaraldehyde, the concentration of glutaraldehyde governs the size and distribution of pores. Increase in concentration of glutaraldehyde causes the growth of pore size and decreases their distribution by restricting the free movement of the polymer chains. As a result, the swelling ratio of the prepared hydrogel also decreases. As the pore size of hydrogel can significantly change gel swelling, it can also affect the properties of hydrogel like drug delivery behavior, enzyme activity, biocompatibility, etc., hence the importance of morphological analysis [101].

Polyacrylamide-chitosan hydrogel, a chitosan-based hydrogel, was found to have the ability to release drug in a sustained manner [96]. The surface and cross-sectional morphology of the freeze-dried hydrogel was examined by SEM which revealed porous nature of the matrix with interconnected channel-like structures. The SEM images also helped to compare the nature of polyacrylamide-chitosan hydrogel over pure chitosan matrices which are fragile and exhibit uncontrollable porosity. The freeze-dried hydrogel showed a faster and extensive swelling which can be explained by the morphological analysis. As the hydrogel has interconnected pores, these channel-like structures can take up the water phase in the matrix by capillary action, and as a result swelling is achieved by fast diffusion of solvent in the matrix. Thus morphological characterization helps to explain various physical properties of hydrogel like hydrophilic nature, hardness, swelling behavior, etc.

In case of gelatin/carboxymethyl-chitosan hybrid hydrogel prepared by radiation-induced-cross-linking intended to be used in tissue engineering where porous
microstructure plays significant influence, SEM images can confirm the nature and interconnected structure of the pores (Fig. 11). By measuring the pore diameter in SEM images, it is also possible to observe the effect of variation in the ratio of gelatin/carboxymethyl-chitosan on pore sizes and the thickness of the wall of prepared hydrogel scaffolds [102].

SEM images also help to explain the effect of pH of the solution on the structure of hydrogel. In a hydrogel system composed of N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) and glycerophosphate (GP), it was found that the structure of the hydrogel changed greatly after dipping in acidic and basic solution [100]. The original HTCC/GP hydrogels had more compact structure due to low crystallinity and high uniformity of HTCC, but after dipping in acidic solution, large pores were observed in the network (Fig. 12a). The reason behind formation of these pores was good hydrophilicity of HTCC, i.e., the cationic quaternized chitosan dissolved quickly in acidic solution. As chitosan is insoluble in basic medium, the structure HTCC/GP hydrogel did not change apparently when dipped in basic solution (Fig. 12b).

The effect of constituent’s molecular weight on the structure of hydrogel can also be determined by analyzing SEM images. An injectable triple cross-linking network

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**Fig. 11** The scanning electron micrographs of the lyophilized gelatin/carboxymethyl-chitosan hydrogels prepared at different ratios of gelatin to carboxymethyl-chitosan. Open and interconnected porous structures are clearly visible in each image. (Reprinted with permission from [102] Copyright © 2010, Elsevier)
hydrogel prepared from chitosan and poly(ethylene glycol) diacrylate (PEGDA) was found to have a highly macroporous, sponge-like structure with average pore size of 20–60 μm [99]. However, changing the molecular weight of one of the constituents PEGDA, it was found that the pore size became larger with the increase of molecular weight of PEGDA. This phenomenon arises because PEGDA with higher molecular weight has better solubility and helps it to stretch sufficiently to create a larger pore size network. Such porous structure of hydrogel is helpful for the delivery of macromolecular compounds as they can diffuse freely into the pores.

In nanocomposite hydrogels like chitosan-iron oxide coated graphene oxide (GIO) nanocomposite hydrogel prepared by gel casting technique, SEM images help to observe the nature of distribution of nanomaterials in the polymeric hydrogel matrix. From SEM images, the chitosan-GIO nanocomposite hydrogel was found to have a compact packing of hydrogel network macrostructures with surface roughness which is the unique morphology of hydrogel prepared by gel casting technique. It is also possible to tune the surface properties like hydrophobicity which can be changed by varying the loading percentage of iron oxide coated graphene oxide nanomaterial in the chitosan matrix by analyzing SEM images [103].

Silver nanoparticles loaded poly(vinyl alcohol)/chitosan hydrogel thin films is another chitosan-based nanocomposite hydrogel prepared via ultraviolet (UV) irradiation [104]. The surface and cross-sectional morphologies of the PVA/chitosan hydrogel (PCA) and the Ag nanoparticles loaded composite (PCA/Ag) were revealed in SEM images. Interconnecting pore structures were observed in the top surface of the PCA thin film with the pore size in the submicron scale (Fig. 13a), but in case of PCA/Ag composite, expanded pore structures were observed with uniform distribution Ag nanoparticles in the composite (Fig. 13b and c).
3.2 Laser Scanning Confocal Microscopy of Chitosan-Based Hydrogels

Although SEM is the most established method to observe the morphology of hydrogel because of its ability to provide structural information in sufficient detail, this technique suffers from some disadvantages of which collapse of pore structure during dehydration leading to volume shrinkage is the most serious one. In the native state, hydrogel contains substantial amount of water which must be removed prior to SEM examination that affects the morphology of hydrogel.

Two common methods used to dehydrate hydrogels prior to examination by SEM are freeze-drying and critical point drying [105]. In case of freeze-drying, if the rate of freezing is too slow, then ice crystals are formed instead of vitreous ice which can cause damage to the sample. In case of critical point drying, the hydrogel may melt if the temperature required to reach supercritical conditions is above the glass transition point of the hydrogel resulting in distortion or destruction of pore structure. Moreover to examine the internal structure, freeze-dried hydrogel has to be fractured which causes further impairments.

Fig. 13 SEM images of the cross section of (a) PCA, (b) and (c) PCA/Ag. (Reprinted with permission from [104] Copyright © 2014, Elsevier)
Laser scanning confocal microscopy (LSCM) is an alternative method to investigate the morphology of hydrogel in its native state, i.e., hydrated state. It is a far simpler and more rapid technique for imaging hydrogels than SEM. LSCM allows to take images of hydrogel without fracturing or cutting the sample [106]. In this method a series of images taken at successive intervals and by magnifying special region of interest can be superimposed to get detailed morphological information. With the help of application software that utilizes a series of successive LSCM images, the three-dimensional nature of hydrogel can also be observed.

The basic principle of LSCM includes labeling the hydrogel sample with a fluorescent dye and excitation of the sample by laser light. The emitted red-shifted light from the fluorescent dye is then detected and used to construct an image. As both labeling and imaging can be carried out in the hydrated state of hydrogel so no dehydration of hydrogel is required, as a result the aforementioned problems encountered in SEM can be avoided [105].

Typical sample preparation for LSCM involves soaking of hydrogel sample in aqueous solution of fluorescent dye, e.g., fluorescein isothiocyanate or rhodamine B isothiocyanate for about 24 h. To remove excess dye, the sample is rinsed off with distilled water for several times. The rinsing operation is carried out in the dark to prevent photobleaching of the dye, and after completion of washing, the sample is usually stored in dark at 4°C [105].

Morphological analysis plays a vital role in design and fabrication of multilayered chitosan hydrogel with oriented structure. Multilayered chitosan hydrogel was prepared by gelation process without any auxiliary cross-linking agent [98]. The effect of concentration of chitosan on hydrogel was observed and found that orientation of layers in hydrogel cannot always be observed. When chitosan concentration was less than 0.5 wt.%, an ordinary random porous 3D network was formed without formation of any layer (Fig. 14a). But when the concentration was higher than 1.0 wt.%, oriented multilayered structure with spatially separated layers was observed (Fig. 14b). As the orientation only observed in hydrogels with relatively high concentration of chitosan, so polymer concentration played an important role in the formation of hydrogel. Again the method of preparation has effects on formation of layers in hydrogel. In LSCM images, the layers are clearer when the hydrogels are prepared by cylindrical mold than single-opening mold. Hydrogel prepared by cylindrical mold showed layers were formed in the form of concentric circles and orientation presented a radial pattern (Fig. 15a and b). It is important to understand such layered-oriented structure and direction of orientation through morphological analysis to control and design the structure of hydrogels.

In case of chitosan/glycerophosphate (GP) hydrogel, a chitosan-based thermo-sensitive hydrogel, LSCM has found to be a valuable technique for imaging hydrogel microstructure which provides both qualitative and quantitative data as well as helps to understand the gelation mechanism [97]. A comparative study of morphology of this hydrogel by analyzing images of two techniques, SEM and LSCM, was also carried out. From LSCM images it was found that chitosan/GP hydrogel had a heterogenous, beaded, open network structure formed by linking polymeric
aggregates in agglomerates and chains, while SEM images failed to detect this fine aggregate structure. The effect of variation of chitosan content in hydrogel was also observed, and it was found from confocal images that the polymer-rich phase increased with increasing chitosan content (Fig. 16).

One of the unique features of LSCM analysis is the visualization of three-dimensional structure of hydrogel. Utilizing a series of successive LSCM images of chitosan/GP hydrogel, the three-dimensional nature of hydrogel was observed which ensured random microstructure in all dimensions (Fig. 17).

**Fig. 14** Morphology of chitosan hydrogel prepared by single-opening mold (a) LSCM image, concentration of chitosan is 0.1 wt.%, cross section (b) LSCM images, concentration of chitosan is 4.0 wt.%, longitudinal section [98]

**Fig. 15** Morphology of chitosan hydrogel prepared by cylindrical mold (a) Orientation in radial pattern, LSCM image, bright field; (b) concentric layers, LSCM image, bright field [98]
Fig. 16  LSCM images of chitosan/GP hydrogel with variations in chitosan content. Inset optically magnified 10X. Chitosan compositions: (a) 0.25 wt.%/vol.%, (b) 0.5 wt.%/vol.%, (c) 0.75 wt.%/vol.%, (d) 1.0 wt.%/vol.%, (e) 1.25 wt.%/vol.%, (f) 1.5 wt.%/vol.%. White areas represent the polymer-rich phase. (Reprinted with permission from [97] Copyright © 2005, Elsevier)

Fig. 17  Three-dimensional visualizations of chitosan/GP gels, using stacked LSCM images. (a) 0.25 wt.%/vol.% chitosan, (b) 1.0 wt.%/vol.% chitosan. (Reprinted with permission from [97] Copyright © 2005, Elsevier)
4 Morphological Characterization of Collagen-Based Hydrogels

Collagen is the most abundant protein in mammals and the main structural component in the connective tissue of extracellular space. Degree of mineralization decides whether collagen tissue would be rigid (bone) or compliant (tendon). Cartilage is a special kind of collagen tissue which inclines from hardness to flexibility. According to Robert H. Bogue, collagen is not a simple anhydrous of gelatin but rather a polarized complex produced by chemical condensation [107]. Collagen becoming a natural product has extensive use in wound healing [108], bone grafting [109], and regeneration of tissue by scaffolding [110].

The water sorption ability and swelling kinetics of a hydrogel depend on the porosity of hydrogels. For instance, to make available space for cell seeding, growth, and proliferation, optimum porosity of hydrogel for tissue regeneration is ranging from 5 μm in neovascularization to 100–350 μm in bone regeneration [111]. Therefore, increment of porosity in hydrogels is regarded as the most important issue in most researchers. It can be achieved in collagen-based hydrogels either by physical or chemical techniques. The chemical techniques may encompass phase-separation, foaming, lyophilization, solvent casting, particulate-leaching, etc. [28], while the physical techniques may include laser sintering [29] and laser-enhanced surface modification [30].

4.1 Scanning Electron Microscopy of Collagen-Based Hydrogels

It is mentioned earlier in this chapter that extensive sample preparations are required for the capturing the SEM images of an object to monitor the surface morphology. A. Pourjavadi et al. observed the porosity of a collagen-based hydrogel which was prepared by chemically modifying collagen with acrylamide (AAm), acrylic acid (AA) \([\text{AAm:AA} = 2.17]\), and \(N, N\)–methylene bis acryl amide (MBA), where grafting was initiated by potassium polysulfate (KSP) and subsequently neutralized by sodium hydroxide [112]. They depicted SEM images in Fig. 18 which showed that the porous structure was more prominent in grafted copolymers due to neutralization after the gel formation. They assume water evaporation resulting from the neutralization heat introduced more in the hydrogels.

Clay-free composite hydrogels usually show higher porosity than its counterparts. It appeared in an investigation of Kabiri et al. that porogen has lost its efficiency in presence of clay while producing porous collagen-based composite incorporating poly (AA-co-potassium acrylate) and kaolin in collagen matrix [113]. They investigated the morphology of superadsobent hydrogel composite with different porogens such acetone, sodium bicarbonate, and acetone-sodium bicarbonate in combined. It was observed that porosity was lowered considerably due to addition of kaolin [113].
The type of clay is also an important factor that influences the porosity of superabsorbent composite. Zhang et al. examined the impact of five different clays including attapulgite, kaolinite, mica, vermiculite, and montmorillonite on absorption rate of a superabsorbent hydrogel composite [114]. It was found montmorillonite-composite displayed the highest swelling capability probably due to its higher porosity in the matrix [114]. Nistor et al. investigated the porosity of superabsorbent hydrogel composite intercalating three types of montmorillonite nanoclays – Dellite® HPS (HPS), Dellite® G67 (G), and Cloisite® 93A (C). Dellite® HPS nanoparticles are natural nanoclays, respectively, a purified montmorillonite unmodified, while Dellite® 67G is a nanoclay deriving from a naturally occurring montmorillonite especially purified and modified with a high content of quaternary ammonium salt (dimethyl dihydrogenated tallow ammonium). Cloisite® 93A nanoparticles are products of Southern Clay Products which are natural montmorillonites modified with a ternary ammonium salt, respectively, methyl dehydrogenated tallow ammonium, for an organic modifier concentration of 95 meq/100 g clay [115]. The SEM images showed in Fig. 19 displays the evidence of disorganized porous structures of the all types of hydrogels, where hybrid hydrogel impregnated with Dellite® HPS showed the largest size of pores of 60 ± 5 μm and hybrid hydrogel impregnated with Dellite® G67 showed the smallest size of pores of 30 ± 10 μm.
From their experiments, Nistor and his coworkers emphasized that the uniform distribution of pores depended on the electrical charge of the nanoclays and types of the ions in the chemical structure of the hydrogel matrix [115].

4.2 Atomic Force Microscopy of Collagen-Based Hydrogels

Atomic force microscopy (AFM) is a powerful tool for surface analysis. To know the surface topography with nano or even atomic resolution AFM has been contributing widely since early 1980 [116]. While the electron microscope provides a
two-dimensional projection or image of a sample, the AFM delivers three-dimensional surface details. In addition, AFM does not require any extensive sample preparation (such as carbon or metal coatings) which is mandatory for SEM or TEM. Above all, high-resolution AFM conveys comparable information in consideration with SEM, TEM, and STM.

In the field of tissue engineering, such as skin burns, wound healing, etc., roughness of the surface plays an important role. For healing of burned tissue, slightly rough surface in the hydrogels is generally desired [117]. As mentioned earlier in the immediate previous section of SEM of superabsorbent hydrogel intercalating montmorillonite nanoclays, Nistor and his coworkers also observed the surface roughness through AFM [115]. In Fig. 20, comparatively even surface was exhibited in case of hydrogel without montmorillonite nanoparticles, while a nodular morphology and extended structure were observed on the surface of hybrid hydrogels. Due to incorporation of Cloisite® 93A, Dellite® HPS, and Dellite® 67G, the average roughness of the hybrid hydrogels increased to 8.14 ± 0.63 nm, 15.64 ± 1.05 nm, and 17.32 ± 0.87 nm, respectively, where surface of the hydrogel without nanoclays found considerably smooth (4.27 ± 0.08 nm). It appeared that the surface morphology depends on nanoparticle type. The selective accession of crowded montmorillonite nanoparticles to the polymer chain (attachment of nanoparticles to the functional groups of polymers) during the synthesis of hybrid hydrogels influenced the roughness texture and uniformity of surface.

![AFM images](image_url)
5 Morphological Characterization of Gelatin-Based Hydrogels

Gelatin is an assortment of peptide and proteins produced by partial hydrolysis of collagen extracted (by chemical denaturation) from the skin, bones, and connective tissues of animals such as domesticated chicken, cattle, pigs, and fish [118]. During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Its chemical composition is, in many aspects, closely similar to that of its parent collagen [119]. Gelatin is generally consist of carbon, 6.8% hydrogen, 17% nitrogen, and 25.2% oxygen [120]. Gelatin has extensive use in food, pharmaceutical, cosmetic, and photographic industries, as it has the distinctive functional properties. In the field of food industry, gelatin is regularly being used in bakery, dairy, beverages, and confectionary for gelling, emulsification, texturization, and stabilization [121]. In the meantime, gelatin is widely being used in drugs encapsulation (both as hard and soft form), ointment filling, wound dressing, plasma expanding, and emulsification in pharmaceutical industries [122]. Moreover, gelatin has been applied as emulsion layer, non-cult layer, and coating layer on the photographic materials [123].

5.1 Scanning Electron Microscopy of Gelatin-Based Hydrogels

As mentioned previously in this chapter, hydrogels can be prepared either using chemical method [28] or physical method [29]. Interconnection of pores cannot be assured in the hydrogels which are generally produced by chemical methods, even the organization of pore size are complicated due to uneven evaporation during drying. Moreover, risk of toxicity and carcinogenicity due to existence of residual solvent discourages hydrogels produced by chemical method for application of public health [124].

In concern with physical well-being, physical approaches of hydrogel preparation are more attractive in opposition to chemical techniques. Physical method like femtosecond laser for modification is a one-step process with precision, non-contiguous to chemicals or solvents, and free from objectionable thermal damage [126]. Daskalova et al. displayed the efficacy of application of femtosecond laser pulses for successful modification of surfaces of gelatin thin films, for creation of micro- and nanoscale structures [125]. Laser used by them was a CPA Ti: sapphire laser (Femtopower Compact pro) emitting at 800 nm central wavelength, with pulse duration of 25 fs, at repetition rate of 1 KHz, and average output power of 800 mW. Condition used for the treatment was air and sample object was placed few mm away to avoid nonlinear optical effect of air. The size of the spot was 182 μm measured by shot diameter regression technique [127]. The films of gelatin were irradiated with various fluences (a stream of particles crossing a unit area, generally represented by F with a unit of Joule per square cm) and various numbers of pulses (unitless and dimensionless parameter generally represented by N). Then the irradiated thin films of gelatin was observed utilizing SEM [125].
Daskalova et al. noticed the formation of the rim surrounding the central area of the spot ($N = 1$) in Fig. 21a and another new rim formation in Fig. 21b due to irradiation of second pulse ($N = 2$). The effect of rim formation was reproducible even when a larger number of pulses was applied on the films exhibited in Fig. 21c–f. The formation of rim is considered as a consequence of the splash of a resolidified molten material generated during the process of laser impact. Due to larger impact of the laser, the formation of a crater in the center started to form at 25 pulses ($N = 25$) and a hole was witnessed at 100 pulses ($N = 100$). Higher degree of magnification of SEM images were showed in Fig. 22a–d where it was observed that porosity of the matrix started to generate (Fig. 22b) at lower pulse ($N = 2$) and became obstructed (Fig. 22d) at higher pulses ($N = 25$). This alteration was explained as melting-freezing or sublimation-deposition of gelatin in the inner irradiated area. This phenomenon assured the researchers to adjust the structure of the gelatin film for their special purposes [125].

Fig. 21 SEM images (2000×) of a surface modification of thin gelatin film irradiated at $\lambda = 800$ nm, $T = 30$ fs laser pulse and laser fluence $F = 2.5$ J/cm$^2$: (a) $N = 1$, (b) $N = 2$, (c) $N = 3$, (d) $N = 4$, (e) $N = 5$, (f) $N = 10$, (g) $N = 25$, (h) $N = 50$, (i) $N = 100$. (Reprinted with permission from [125] Copyright © 2013, Elsevier)
Hydrogels are the three-dimensional polymer networks that are swollen by trapping large amounts of water. Those networks are formed by molecular self-assembly through covalent, ionic, or hydrogen bonds [128]. According to the sources, hydrogel can be classified into those formed from synthetic polymers and those formed from natural polymers. Both types of hydrogels have versatile applications in food products to medical purposes. Owing to the adjustable mechanical properties, ability for photopolymerization, and easy control of scaffold architecture and chemical compositions, the demand of synthetic polymer hydrogel has increased exponentially [129]. Among the vast polymer application, only limited polymers have the ability to form hydrogel networks. Some of the synthetic polymer-based hydrogels
have been reported such as poly(ethylene glycol) [37], poly(vinyl alcohol) [33], poly(amide-amine) [36], poly(N-isopropylacrylamide) [34], polyacrylamide [38], and poly(acrylic acid) [35] and their copolymers [130]. The physical characteristics such as amount of void space, pore size, wall thickness, etc. vary from polymer to polymer, fillers as well as preparation method. In general, the pore size increases with an increasing swelling ratio of the hydrogels [131]. The sampling for morphology analysis of synthetic polymer was similar to natural polymers.

Polyaniline-polyvinyl alcohol (PANI-PVA) hydrogel, a PVA-based hydrogel, was reported as self-supported electrode for supercapacitors [132]. The aniline monomer was dissolved in acidic PVA solution and performed in situ polymerization, accompanied with freezing-thawing gelation of PVA. The morphology of this composite hydrogel was observed by Field Emission Scanning Electron Microscopy (FESEM), and the FE-SEM images of PVA and PANI-PVA hydrogel are presented in Fig. 23. It is revealed that pure freeze-dried PVA hydrogel shows nanoporous morphology with pore size ranging from several micrometers to nanometers (Fig. 23a). In contrary, incorporated PANI/PVA hydrogel maintains the nanoporous PVA chain structure with some nanoaggregates of nanorods, nanosheets, and nanoparticles formed from PANI chains (Fig. 23b). The uniformly distributed PANI nanoaggregates can provide ion/electron transmission channel for electrochemical reactions within the PANI/PVA hydrogel and enhances the suitability as a supercapacitors.

Xu et al. have prepared controlled porous structure PVA-based hydrogels by freezing technique. The influence of poly(ethylene glycol) (PEG) on the microstructure was investigated by SEM and found that the porosity of hydrogels was significantly increased with PEG incorporated and the pore size increased with the increasing of PEG molecular weight [133]. Shi et al. have reported contrary results in PVP-PVA composite hydrogel. The results showed that with the increasing polymerization degree and polymer concentration of PVA, the network structure of

Fig. 23 FE-SEM images of (a) PVA and (b) PANI-PVA hydrogel. (Reprinted with permission from [132] Copyright © 2016, Springer Science+Business Media New York)
hydrogels became denser and porosity significantly decreased [134]. Shi et al. have also synthesized and characterized PVP/PVA hydrogel for the applications of articular cartilage replacements [135]. The SEM images of PVA/PVP hydrogels before and after swelling in non-osmotic and osmotic solutions for 28 days are presented in Fig. 24. The SEM images revealed that all hydrogel samples showed three-dimensional porous network structures. But after immersing the hydrogel in osmotic solution, the microstructure became dense due to hydrogel deswelling in osmotic solution.

Bhowmick and Koul synthesized silver nanoparticles (AgNPs) loaded antimicrobial hydrogel PVA dressing scaffold by using freeze-thaw method [136]. The morphology of PVA hydrogel and AgNPs loaded PVA hydrogels had been characterized by scanning electron microscopy (SEM) at 5000 magnification. The SEM micrograph of both hydrogel shows a microporous web-like structure (Fig. 25). No
segregation of AgNPs in hydrogel was observed and indicated strong interaction between Ag and polymers.

Yu et al. prepared silver nanoparticles incorporated poly(vinyl alcohol)/poly(N-vinyl pyrrolidone) (PVA-PVP) hydrogels by repeated freezing-thawing treatment [137]. The surface and cross-sectional morphologies of Ag/PVA-PVP composite hydrogels were investigated by SEM; both PVA-PVP and Ag/PVA-PVP hydrogels showed porous dimensional network structure. No distinguished difference was found among the hydrogels with different silver contents due to stable network structure within hydrogel and the strong interaction between the silver particles and the PVA and PVP molecules. Owing to excellent antibacterial ability, superb water retention ability and good oxygen transportation capability, Ag/PVA-PVP may be used a potential wound care dressing.

Hu et al. have prepared polyvinyl alcohol/carbon dot (PVA/C-dot) hydrogel by freeze-thaw method, and Ag nanoparticles was simply introduced to enhance the antimicrobial activity and enlarge their application potential in medical field [138]. The morphology of this composite hydrogel was investigated by SEM and observed that nanoparticles were uniformly dispersed in PVA/C-dot-Ag hydrogel. The mapping image also showed that the Ag element was uniformly dispersed in PVA/C-dot-Ag hydrogel, indicating uniform dispersion of Ag nanoparticles in PVA/C-dot-Ag hydrogel.

Chen et al. have reported contrary morphology of graphene oxide (GO)-reinforced PVA hydrogels with compared other inorganic nano fillers PVA hydrogel [139]. They synthesized inorganic/organic interpenetrating network (IPN) hydrogels using a freeze-thaw method with a modified GO which was cross-linked by β-cyclodextrin aldehyde (β-CD-DA) to form an inorganic GO network (β-GO). The structure of the hydrogels was investigated by scanning electron microscopy,
and the SEM images of PVA hydrogel, β-GO (1, 2, and 3 mg/ml)/PVA hydrogel and pristine GO (1, 2, and 3 mg/ml)/PVA hydrogels are shown in Fig. 26. In the SEM micrograph, PVA hydrogel shows porous structures. The other hydrogels displayed dense structures regardless of whether GO or β-GO was present. The incorporation of GO can efficiently fill pores in pure PVA hydrogels.

Poly(N-isopropylacrylamide) (PNIPAAm)/poly(vinyl alcohol) (PVA), a PVA-based temperature-sensitive semi-interpenetrating polymeric network (IPN) hydrogel, was found to have the fastest temperature responsive properties [140]. The PNIPAAm networks were cross-linked by N,N'-methylenebisacrylamide (MBAAm) in the presence of linear PVA. The prepared semi-IPN hydrogels were characterized for their morphologies by scanning electron microscopy, and the SEM micrograph of the freeze-dried hydrogels is shown in Fig. 27. It was found that the hydrogel morphologies were dependent on the feed compositions of PNIPAAm and PVA. The morphology of conventional PNIPAAm exhibited a homogeneous, dense architecture, while IPN hydrogels had uneven and porous structures. In addition, the pore size seemed to decrease with increasing PVA content. Because of the fraction of the cross-linked PNIPAAm networks decreased with decreasing the feed ratio of PNIPAAm and MBAAm from PNIPAAm to IPN1–3, so the supporting force of the cross-linked networks correspondingly decreased, resulting in the shrinking and partial collapse of the bulky networks during the freeze-drying process. In other words, the higher the PVA content, the more pores can be observed. This is possibly ascribed to the increase in the swelling ratio and water content for IPN hydrogels due to the incorporation of hydrophilic PVA, leading to a reduction in the polymer fraction for IPN hydrogels.

Poly(vinyl alcohol)-polyetheretherketone/poly(vinyl alcohol)-β-tricalcium phosphate (PVA-PEEK/PVA-β-TCP) bilayered hydrogels have been investigated by Li et al. [141]. These bilayered hydrogels were developed by freezing-thawing with biomimetic properties for articular cartilage and subchondral bone. The bilayered hydrogel microarchitecture consists of a highly porous and dense structure, and the morphology of the resulting hydrogels was analyzed by scanning electron

Fig. 26 The SEM micrograph of PVA and GO-loaded PVA hydrogels. (Reprinted with permission from [139] Copyright © 2016, Elsevier)
The microstructure of the pure PVA bilayered hydrogels is homogeneous. The typical micrographs of the cross sections of A-K/A-P bilayered hydrogel showed etched features and rough structures with many granules present on the surface. Furthermore, the lower porous layers have an internal three-dimensional structure with lots of micropores on the surface and pore sizes recommended for cartilage tissue engineering scaffolds. These structures indicate that a good bonding exist between the two layers, which is known to be a necessary condition to assure a good integrity and functionality of the osteochondral construction. The lower layer of porous pure PVA bilayered hydrogels had showed a smooth porous network structure, whereas the A-K/A-P was a relatively homogeneous and porous structure with good pore connectivity. The structures of A-K and A-P were not significantly different from the composite bilayered hydrogel, and the formation of the bilayered structure did not affect the pore size, the porosity, and the microstructures.

Zhou and Li synthesized temperature-sensitive poly(N-isopropylacrylamide) (PNIPAAm)/poly(ethylene glycol)s (PEGs) hydrogels [142]. The effect of molecular weight (2000–6000) and PEG content on the morphology of these hydrogels were analyzed with scanning electron microscopy. The micrograph revealed that the
PEG-modified PNIPAAm hydrogels have more porous networks and the surfaces are looser than those of the conventional hydrogels. In addition, the average pore size of the PEG-modified hydrogels becomes larger with higher-molecular-weight PEGs and higher PEG content.

Fig. 28  SEM images of surfaces for PVA-PEEK (A-K) layer (a) and PVA-β-TCP (A-P) porous layer (b), fracture surfaces for bilayered PVA/PVA (A/A) (c, e) and PVA-PEEK/PVA-b-TCP (A-K/A-P) (d, f). (Reprinted with permission from [141] Copyright © 2016, Elsevier)
Comelli et al. investigated the PNIPAAm-PEG hydrogels after solvent immersion and have found interesting results [143]. The hydrogels were immersed in phosphate-buffered saline (PBS) for 14 days at 37 °C and lyophilized after immersion. The resulting polymeric hydrogel morphology was evaluated using scanning electron microscopy (SEM). The SEM micrograph of PNIPAAm-PEG scaffold immersed in PBS is shown in Fig. 29; the scaffold exhibits a macroporous structure, but the pores do not appear to be interconnected, as is commonly observed in polymeric hydrogels.

The morphology of aminated hyaluronic acid-g-poly(N-isopropylacrylamide) (AHA-g-PNIPAAm) thermosensitive copolymer hydrogels has been reported by scanning electron microscopy [144]. These hydrogels were synthesized by coupling carboxylic end-capped PNIPAAm (PNIPAAm-COOH) to AHA through amide bond linkages. The SEM images of freeze-dried AHA-g-PNIPAAm hydrogels before and after PBS incubation at 37 °C were shown in Fig. 30. The initial AHA-g-PNIPAAm-28 (28% PNIAAm loaded) and AHA-g-PNIPAAm-53 (53% PNIAAm loaded) copolymer hydrogels displayed a continuous and porous structure. The pore diameter of the AHA-g-PNIPAAm-28 copolymer hydrogel is in the range of 3–20 μm, compared to a diameter of 1–10 μm pores for the AHA-g-PNIPAAm-53 copolymer hydrogel. This difference in pore size indicates that a higher grafting ratio of PNIPAAm results in the formation of smaller pore diameters and tighter network. After 21 days of incubation, the structure of AHA-g-PNIPAAm-28 hydrogel was partially changed, but there was no significant influence brought to the AHA-g-PNIPAAm-53 hydrogels structures.

The effect of enzymatic degradation on the morphology of AHA-g-PNIPAAm hydrogel was also investigated, and the hydrogels were subjected to enzymatic degradation in 100 U/mL hyaluronidase/PBS at 37 °C for 7 days and 21 days. After degradation, the AHA-g-PNIPAAm-28 hydrogel resulted in larger pore
diameters (Fig. 31), which is likely due to the mass released from the gel matrix. Compared to AHA-g-PNIPAAm-28, the AHA-g-PNIPAAm-53 hydrogel showed a stable morphological structure during enzymatic degradation, and the pore diameters changed only slightly (Fig. 31), although the weight loss ratios significantly increased from 7 days to 21 days.

Li investigated the morphology of dry poly(acrylic acid-acrylamide-methacrylate)-amylose hydrogel by using field emission scanning microscopy (FE-SEM). The micrograph showed that the hydrophilic amylose homogeneously dispersed in poly(acrylic acid-acrylamide-methacrylate) hydrogel and gave large porous network structure. This highly porous (pore size ~ 100 μm) structure hydrogel may be suitable adsorbent for crystal violet [145].

Jin et al. have developed spherically shaped semi-interpenetrating network (semi-IPN) poly(N-vinylpyrrolidone) (PVP)/poly(acrylic acid) (PAA) hydrogel [146]. The morphology of PVP and semi-IPN hydrogels was investigated by scanning electron microscopy (SEM). The cross-sectional morphology of dried PVP gels swollen completely in two buffer solutions of pH 2.07 and 10.98 are shown in Fig. 32 and observed that the two hydrogels had similar surface morphology with the larger three-dimensional pores corresponding to similar swelling capacity and very thin pores wall. In contrast, buffer swollen semi-IPN hydrogels had different morphologies. In comparison with the morphologies of PVP hydrogels, the semi-IPN hydrogel had two peculiar features: one was the difference of diameter of the

**Fig. 30** SEM images to show the internal structures of AHA-g-PNIPAAm-28 (a–c) and AHA-g-PNIPAAm-53 (d–f) hydrogels before and after incubation in PBS at 37 °C for different times. (b, e and c, f) are 7 days and 21 days, respectively. (Reprinted with permission from [144] Copyright © 2009, Elsevier)
Macro pores of the semi-IPN hydrogels swollen in two type solutions. The SEM images shows the smaller three-dimensional net-hole due to the rather compact structure of the semi-IPN hydrogel swollen in buffer solution of pH 2.07 and the larger three-dimensional net-hole of the semi-IPN hydrogel swollen in buffer solution of pH 10.98 (Fig. 32).

The effects of saline water and buffer solutions in poly(vinyl alcohol) (PVA)/poly(acrylic acid) (PAA) hydrogels on morphology have been reported [147]. The PVA/PAA hydrogels was hydrated in saline water at pH 2.8 and pH 5.8, and network structure was observed. A strong effect of the pH was observed in the pore size and inner structure of hydrogels. At pH 2.8, it displayed irregular pore shapes with variable sizes and at pH 5.8, expanded porous structure due to larger swelling was observed.
Morphological characterization of hydrogel reveals essential information regarding the surface of hydrogel as well as the size and shape of the pores in the structure of the hydrogel. Moreover, these characterization information are vital in determining the feasibility of the intended application of the synthesized hydrogel. Morphological properties like porosity of hydrogels, typically analyzed by scanning electron microscope images, are important parameters in deciding the effectiveness of hydrogels in application such as drug delivery system. Not only that, laser scanning confocal microscopy has been successfully used to analyze multilayered hydrogel with oriented structure and in cases where SEM is not viable due to risk of structural collapse during dehydration in SEM technique. As a result, these morphological

![Fig. 32](image)

**Fig. 32** The SEM micrographs of the hydrogels. (a) The PVP hydrogel swelled in buffer solutions of pH 2.07, (b) the PVP hydrogel swelled in buffer solutions of pH 10.98, (c) the semi-IPN hydrogel swelled in buffer solutions of pH 2.07, (d) the semi-IPN hydrogel swelled in buffer solutions of pH 10.98. (Reprinted with permission from [146] Copyright © 2006, Elsevier)
techniques have been extensively used in designing hydrogels from both cellulose and other biopolymers.

Hydrogels have become a tremendously popular field in research fraternity. The scope it offers far outweighs the trivial disadvantages it brings in applications. Advantages, such as low cost, non-toxicity, hydrophilicity, biodegradability, transparency, and biocompatibility, which biopolymers, particularly cellulose-based hydrogels, bring in, are unmatched and drawing more attention toward this field. In fact, more and more avenues – drug delivery system, water purification adsorbent, chromatographic supports, and biosensors – are being ventured into to unfurl the true prospect of hydrogels. But there are still plethora of opportunities available to look into for further investigation in the field of cellulose and other biopolymer-based hydrogels. Because of these unrivaled attributes and exponentially growing interest, cellulose-based hydrogel superabsorbents have the potential to become a sustainable and worthy replacement of synthetic polymer-based hydrogels.

References


