



Condensed Matter and Interphases (Kondensirovannyye sredy i mezhfaznyye granitsy)

Review

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Modern Approaches to the Medical Use of pH- and Temperature-Sensitive Copolymer Hydrogels (Review)

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Abstract

This article provides the review of the medical use of pH- and temperature-sensitive polymer hydrogels. Such polymers are characterised by their thermal and pH sensitivity in aqueous solutions at the functioning temperature of living organisms and can react to the slightest changes in environmental conditions. Due to these properties, they are called stimuli-sensitive polymers. This response to an external stimulus occurs due to the amphiphilicity (diphilicity) of these (co)polymers. The term hydrogels includes several concepts of macrogels and microgels. Microgels, unlike macrogels, are polymer particles dispersed in a liquid and are nano- or micro-objects. The review presents studies reflecting the main methods of obtaining such polymeric materials, including precipitation polymerisation, as the main, simplest, and most accessible method for mini-emulsion polymerisation, microfluidics, and layer-by-layer adsorption of polyelectrolytes. Such systems will undoubtedly be promising for use in biotechnology and medicine due to the fact that they are liquid-swollen particles capable of binding and carrying various low to high molecular weight substances. It is also important that slight heating and cooling or a slight change in the pH of the medium shifts the system from a homogeneous to a heterogeneous state and vice versa. This provides the opportunity to use these polymers as a means of targeted drug delivery, thereby reducing the negative effect of toxic substances used for treatment on the entire body and directing the action to a specific point. In addition, such polymers can be used to create smart coatings of implanted materials, as well as an artificial matrix for cell and tissue regeneration, contributing to a significant increase in the survival rate and regeneration rate of cells and tissues.

Keywords: hydrogel, microgel, N-isopropylacrylamide, thermal sensitivity, pH sensitivity, heterophase polymerisation.

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1. Introduction

In recent decades, a new scientific direction has emerged at the intersection of polymer chemistry, nanotechnology, biology, pharmaceuticals, biotechnology, medicine, and it is constantly attracting increased attention, associated with the so-called «smart» or «intelligent» materials [1]. Smart materials based on water-soluble polymers attract significant scientific and practical interest. This is due to the fact that they have a number of unique properties: temperature-, pH-sensitivity, structure diphilicity, etc. In this regard, they are called stimuli-sensitive polymers, since they can respond to external environmental influences [2-5]. The reaction to external influence occurs due to the amphiphilicity (diphilicity) of the (co) polymers. Usually, monomers with a structure containing both hydrophilic and hydrophobic segments are used. Such substances are now being actively used as dispersants, emulsifiers, solubilisers, in cosmetology, for the isolation of drugs, etc. [6, 7].

Among smart polymers there are representatives that can respond to changes in temperature, pH, ionic strength, the content of inorganic, organic substances and high molecular weight compounds of various natures, light intensity, electric field strength, etc. Methods for the synthesis of such hydrogels (precipitation polymerisation, emulsion polymerisation) are often simple and extremely easy to scale up to pilot production, and the synthesized particles obtained by these methods will have a rather narrow size distribution.

These systems will undoubtedly be promising for their use in biotechnology and medicine. They are a very simple tools for managing systems. Slight heating and cooling or a slight change in the pH of the medium leads to a transition from a homogeneous system to a heterogeneous one and vice versa, significantly changing the swelling capacity of the hydrogels. Such polymers are already used for the concentration and purification of biologically active substances or for the fixation of biocatalysts.

Recently, methods have been developed for the preparation of such hydrogels from biodegradable and non-toxic polymers [8]. The term hydrogels includes both macro- and microgels. The latter differ in that the polymer

particles are dispersed in a liquid and are nano- or micro-objects. Due to the fact that the microgel is a liquid-swollen particle, they can bind and transport inside various substances of low to high molecular weight. This fact suggests that such systems are ideal for the delivery of drugs. It is possible to create a system with precisely specified parameters at which it will operate effectively, achieving accurate drug delivery to the affected cells and tissues by adjusting the size of particles during synthesis or by the external action of the environment (temperature, pH), and by introducing various monomers, regulating the sensitivity and decomposition rate into the composition of the microgel. Additionally, hydrogels also offer great opportunities for regenerative medicine: surfaces coated with such polymers can be used for the growth of cells with the formation of tissues, which can be used either as external coatings for biomaterials for implantation, or for use in the regeneration of various parts of the body.

2. Drug delivery systems

Due to the cross-linked structure of hydrogels containing up to 90% liquid in their pores, they represent a new class of delivery systems for a wide range of different drugs [9]. The indisputable advantages of microgels include the ability to control their composition and properties during synthesis by using various approaches. Microgels with similar chemical compositions can often be obtained in a wide range of sizes from several tens of nanometres up to hundreds of micrometers (depending on the preparation method or the composition of the reaction mixture). By varying the degree of cross-linking of microgels, it is possible to change the pore size inside them, which can be used for the controlled release of the drug loaded into the microgel.

The use of numerous bioconjugation reactions allows the functionalisation of microgels, which is necessary for the transition from “passive” to “active” delivery [10].

The cross-linked structure of microgels provides opportunities for the creation of hybrid nanobiomaterials capable of combining such important biomedical applications as targeted delivery and visualization (the very popular «theranostics» principle [11], derived

from the combination of the words therapy and diagnostics) or targeted delivery and the possibility of local warming up (which is used for hyperthermia therapy with tumours).

In contrast to liposomes, which have been well studied over the past decades and are now widely used in targeted drug delivery therapy, microgels are a less studied and broader class of delivery containers. The most popular are microgels based on polysaccharides: chitosan, dextran, cellulose, and others. [12, 13]. The use of protein-based microgels for targeted delivery is less preferable, since they are more immunogenic; in turn, the use of microgels based on human (albumin) or non-immunogenic proteins (collagen, gelatin) opens up possibilities for the simple synthesis of biodegradable drug carriers [14]. Microgels based on natural polymers have high biocompatibility and extremely low toxicity, but they can be recognised by the components of the immune system and excreted from the body. In turn, synthetic polymers, especially those containing hydrophilic polyethylene glycol or polyglycerol macromonomers have extremely high stability in solution and reduced sorption of proteins, which makes them analogues of «*stealth*»-liposomes. Among synthetic polymers, the overwhelming majority of studies were devoted to microgels based on poly(N-isopropylacrylamide) PNIPAAm [15]. The reasons for this are the ease of preparation and monodispersity of microgels synthesized by the precipitation polymerisation mechanism in an aqueous solution. Among other polymers of targeted delivery using microgels, polymers based on 2-hydroxyethyl methacrylate (HEMA) and oligoethylene glycol methacrylate (OEGMA) are widely used [14].

The possibility to load significant amounts of drug into a microgel and the efficient and controlled release of the loaded drug is important for drug delivery. This requires the binding of the drug to the microgel. This is usually achieved due to hydrophobic interactions (low solubility of the drug in water), but it has been shown that hydrophobic interactions are not enough for the achievement of high (20% or more) degrees of loading with the drug and other types of interactions, hydrogen bonds or electrostatic attraction are required [16].

In most studies, microgels are used to deliver cytostatics: doxorubicin, daunorubicin, cisplatin, and methotrexate [9]. In these studies, microgels are analogues of the liposomal formulations of the mentioned drugs, and the effects observed during their use is often similar. However, the use of microgels open up a wider range of possibilities for the delivery of hydrophilic drugs with a significant number of charged groups. In this case, the binding between the drug and the microgel is carried out due to numerous ionic interactions between the opposite charges of the drug and the hydrogel. This approach was successfully applied in studies based on the delivery of nucleotide triphosphate analogues [17].

The use of ionic interactions for the production of drug-microgel complex is extremely important for biomolecules: nucleic acids and proteins. Microgels can very effectively act as containers for the delivery of biomolecules such as proteins and peptides [18], which makes their use a very promising direction for the delivery vector of drugs.

The use of microgels allows avoiding the side effects of cytostatics, reducing their general toxicity and nephrotoxicity, and contributing to the improvement of the condition of animals in *in vivo* models. Now, the study of targeted drug delivery is one of the most rapidly developing directions and obviously, other drugs will be used for introduction via microgels.

The release of the drug is also crucial for the effective action of the microgel. In many cases, the release of the drug occurs spontaneously, due to diffusion or ion exchange. The use of temperature-sensitive, photosensitive, pH-sensitive polymers allows the controlled release of the drug, providing the selectivity of action on tumours [19].

The environment of a tumour often has a more acidic pH, due to its high metabolic activity and poor development of its lymphatic vessels. pH-dependent swelling of microgels is a universal mechanism of drug release from a microgel, which has been widely investigated in numerous studies. An interesting approach is also pH-dependent swelling leading to the release of functional groups on the microgel surface, which are hidden at a normal pH of 7.4.

In this case, functional groups can be both ligands to receptors on the surface of cancer cells and viral peptides (TAT peptide, etc.), providing an effective pH-dependent penetration of microgels into cells [20].

There are several methods used for the production of biodegradable microgels (8). First, degradable polymers can be used. One example is certain natural biopolymers: polysaccharides and their derivatives, collagen cross-linked with glutaric dialdehyde and many others, as well as widely used polymers based on a copolymer of lactic and glycolic acid (PLGA) or their derivatives. On the other hand, the degradation of the microgel into monomers in the body is not necessary. The polymer itself may be indestructible, but contain crosslinks that can decompose in the body spontaneously (often contain bonds unstable in the acidic pH range, observed in cell lysosomes): β -thiopropionate bonds, methacrylic esters of PLGA, orthoesters, or under the action of certain enzymes. The latter can be based on derivatives of peptides specifically recognised by metalloproteinases.

One of the simplest and most well-studied methods for achieving the degradability of a polymer is the introduction of a crosslinking agent containing a disulphide bond. Often, the synthesis reaction is a radical polymerisation – RAFT polymerisation (*Reversible Addition-Fragmentation chain Transfer*) [21]. The use of RAFT allows more precise control over the composition and properties of synthesised microgels. Disulphide bonds can be easily reduced in a cell's cytoplasm due to the presence of a reduction system, using the glutathione reductase enzyme and the tripeptide glutathione substrate that is present in cells at a concentration of about 5 mM. This system effectively reduces -S-S- bonds due to the disulphide exchange.

Microgels in a swollen state in aqueous solutions are of particular interest. This group of microgels is also often referred to as hydrogels. Hydrogels attract great interest due to their unique physicochemical properties and the huge number of practical applications from photonic crystals and microlenses to containers for drug delivery [22]. Thus, microgels represent an extremely wide class of materials with various compositions, sizes, and morphologies.

According to their method of preparation and the nature of the crosslinking, microgels are often subdivided into physically and chemically crosslinked types [23]. The physical microgels include gels, represented by polymer networks interconnected due to the interlacing of individual macromolecules and/or numerous non-covalent interactions existing between polymer chains. The attractive forces, holding the chains together are hydrogen bonds, van der Waals, electrostatic, or hydrophobic interactions. Thus, such microgels can be reversibly dissolved under certain conditions that weaken these interactions (changes of pH, ionic strength, or the addition of chaotropic reagents).

Another class of hydrogels are chemically crosslinked gels. These microgels are highly stable due to the presence of covalent bonds connecting polymer chains in the microgel network. The main method for obtaining such microgels is polymerisation using polyfunctional crosslinking monomers. At the moment, microgels include a huge variety of polymer particles with different properties. The differences in properties lead to the possibility of classifying certain microgels by their type of sensitivity. Sensitivity is the ability to change physical and chemical properties under the influence of various external parameters.

The most common of these parameters are temperature, pH, ionic strength, light intensity, electromagnetic radiation, and even some simple organic molecules [24]. Most often, microgels undergo a change in their volume, which can be used in many fields of science, such as biotechnology and biomedicine [25].

In addition to the classification according to the sensitivity type, there is a classification according to the method of production. At the moment, there are many different methods with their own advantages and disadvantages. Mini-emulsion copolymerisation of water-soluble monomers in immiscible organic solvents in the presence of surfactants allowing strictly control the composition of the obtained microgels. Using this method it is easy to synthesize microgels with water-soluble macromolecules (proteins, etc.) included in them, as well as nanoparticles (Fe_3O_4 , Pd, Ag, CdSe). The disadvantages of the mini-emulsion polymerisation method [26] include the need to use an external dispersing action, which

often leads to the destruction of biomolecules included in the microgel network.

Microfluidics is a unique method for the synthesis of microgels, which allows the synthesis of polymer particles from 1 to 200 μm , with the narrowest possible size distribution. This is achieved due to the controlled disintegration of the jet of one phase in the medium of the other phase. The special geometry of the channel and the ability to generate different types of drops allow obtaining particles of various shapes, and the channel cross-section diameter strictly determines the range of admissible particle sizes [27]. The particle size of such microgels also depends on the flow rate of the dispersion phase. The advantages of this method include control over the morphology and structure of the resulting particles. Microfluidics allows synthesizing Janus particles (a type of multifunctional micro- or nanosized particles consisting of two or more parts of different chemical composition and/or shape, with different properties) under very mild conditions [28], microgels with particles of various shapes, as well as layered structures by polymerisation of drop-in-drop structures [29], which, in turn, makes it possible to obtain hybrid particles, including those containing living cells inside [30]. The disadvantages of this method include very low productivity, high cost, and the size range of synthesized microgels from few to hundreds of micrometres.

A fundamentally different approach, similar to microfluidics, was developed by *De Simone*. This method is unique, since it allows obtaining polymer particles ranging in size from several tens of nanometres to several microns. This method [31], named “PRINT” (*Particle Replication In Non-wetting Templates*), is a variant of imprint lithography using elastomeric moulds coated with a hydrophobic perfluoropolymer. A solution of monomers or macromonomers in water is placed between two hydrophobic surfaces, a polymerisation or polycondensation process is carried out, after which the surfaces can be easily separated from each other, and the particles can be removed from the mould. This method has undeniable advantages such as strict control of particle size, shape, and composition, and surface functionality. The mild synthesis conditions allow the introduction of unstable compounds and

biomolecules into the microgel system without losing their functionality.

Layer-by-layer adsorption of a polyelectrolyte is one of the most common methods for producing capsules and microgels. *Decher* created the generation of multilayer films on the surface by successive adsorption of cationic and anionic polyelectrolytes [32]. This approach can be used to coat microgels or nanoparticles with polyelectrolytes. For instance, *Sauzedde* [33, 34] described an interesting procedure in which anionic iron oxide nanoparticles (about 10 nm) were adsorbed by cationic microgels. Furthermore, the polymer containing carboxyl groups formed a coating on the surface of the initial microgels, surrounding them with its shell. The approach combining two processes: controlled self-assembly of polymer micelles with the subsequent covalent crosslinking, which provides tremendous opportunities for large-scale production of microgels with a controlled structure. This method allows obtaining a wide range of microgels with various structures by simple selection of solvents and counterions. Using this method spheres, ellipses, and even toroids were obtained [19]. The disadvantages of this method include the need to use monodisperse polymers with a strictly controlled structure and molecular weight, as well as the multi-stage synthesis from monomers to final products.

One of the most attractive approaches to the synthesis of microgels is the use of precipitation polymerisation [35]. The indisputable advantages of this method include the single-stage operation, ease of scaling, high productivity, and the use of water as a solvent («green chemistry»). The composition can be controlled by introducing various monomers capable of providing the desired properties to the microgels. A feature of this process is that during the polymerisation, using a monomer forming a temperature-sensitive polymer, nucleus particles are formed almost simultaneously throughout the volume.

This, in turn, leads to an unusually high monodispersity of microgels for free radical polymerisation. The disadvantages of this reaction include the need to carry out the reaction under heat (usually about 70 °C) and in the presence of free radicals, which excludes the possibility of the direct introduction of sensitive

reagents and biomolecules into the reaction; however, this can be effectively carried out after the synthesis and purification of microgels.

3. Functional biomaterials

The most obvious application of temperature-sensitive hydrogels is culture dishes with layers of PNIPAAm of various thickness and density on the bottom [36], allowing to the adhesion and detachment of cells to be controlled by changing the temperature, forming a cell monolayer detachable from the substrate. One of advantages is that the temperature-sensitive hydrogel remaining on its basal surface [37] acts as a cell glue at body temperature, and the cell layers can be easily transplanted by simply placing the cell layer on the affected area without sutures or other methods of fixation. In addition, cell layers remain in place after transplantation, while cells transplanted as part of an injectable cell suspension have a tendency to migrate [38].

Various types of culture dishes were investigated for the efficient production of cell layers, which led to the emergence of new biomedical technologies (Table 1).

In addition, the cellular activity of monolayers separated from the substrate by simply lowering the temperature can exceed that of cells mobilised by digestive enzymes (trypsin) and emulsifiers (EDTA/EGTA). Thus, the secretion of certain cytokines by the cells of the cell layer may be higher than that of the cell suspension, which can have therapeutic effects.

While exploring the potential use of microgels as coatings for biomaterials, *Gan* and *Lyon* investigated temperature-sensitive PNIPAAm nanoparticles grafted with polyethylene glycol (PEG), obtained by free radical precipitation copolymerisation with PEG monomethyl ether monomethacrylate ($M_w = 1$ kDa) [48]. The solution to the problems of a wide particle size distribution, expansion of the volumetric phase transition of microgels, and a shift in the phase transition temperature to a higher temperature range due to the presence of PEG, was determined to be a two-stage method of precipitation polymerisation. As a result, PEG chains were localised at the outer boundary of the particles. Consistent with numerous previous studies on PEG grafting on both macroscopic surfaces

and particles/macromolecules [49, 50], it was found that protein adsorption on the microgel is suppressed by the incorporation of PEG into the particles, especially when these chains are located in the microgel shell.

Both protein adsorption studies and ^1H NMR showed that the PEG side chains stretch outward from the surface of the particles and that the particles break down at temperatures above the phase transition temperature. Interestingly, similar effects were observed for particles where PEG chains are localised in the particle core. This suggests that PEG grafts could penetrate through the PNIPAAm shell when it is in its phase-separated state.

Similarly, *Nolan* et al. investigated the phase transition and protein adsorption for PNIPAAm microgel particles cross-linked with PEG diacrylates with different ratios and different chain lengths [51]. Based on the method of light scattering, an increase in the temperature and the magnitude of the phase transition with an increase in the concentration of the PEG crosslinking agent included in the microgels was found. Qualitative differences in particle density using centrifugation showed that the obtained microgel networks are denser with a higher PEG concentration. Based on the studies of NMR spectroscopy, it was concluded that the longer cross-links of PEG protrude from the dense globular network, leading to a decrease in non-specific protein adsorption with increasing chain length and PEG content. Similarly, surface-bound microgels containing longer PEG chains showed the absence of fouling and resistance to cell adhesion in serum-containing media.

A similar suppression of protein adsorption and cell adhesion was observed by *Scott* et al. for microgel aggregates formed by octavinyl sulfone, modified by PEG and bovine serum albumin [52]. Considering the geometry-independent surface modification, PEGylated microgels are of potential interest in areas of science requiring antifouling coatings.

South et al. investigated the use of centrifugal aggregation of microgel films for further improvement of the performance of microgel-based coatings by increasing their surface density [53]. In this case, films formed from microgel particles were obtained either by centrifugation

Table 1. Typical threads of cell sheets in restorative therapy implementation

Therapeutic Uses	Cells	Method for creating cell layers	Sources
Corneal plastic	Oral mucosa epithelium	Using temperature-sensitive well plate inserts for culture plates and 3T3 feeder cells treated with mitomycin C.	[39]
Elimination of intraoperative air leak syndrome	Dermal fibroblasts	Using temperature-sensitive culture dishes, transplantation of bilayer cell layers.	[40]
Periodontal regeneration	Periodontal ligament derived cells	Using temperature-sensitive culture dishes, three-layer cell layers with the addition of woven polyglycolic material; bone defects are filled with porous β -tricalcium phosphate.	[41]
Treatment of dilated cardiomyopathy	Myoblasts of striped muscles	Using temperature-sensitive culture dishes, transplantation of cell multilayers	[42]
Prevention of stricture formation after endoscopic excision of the esophageal submucosa	Epithelial cells of the oral mucosa	Using temperature-sensitive well plate inserts for culture plates and endoscopic applications.	[43]
Cartilage restoration	Chondrocytes from knee joints	Using temperature-sensitive culture dishes, transplantation of cell multilayers.	[44, 45]
Prevention of intrauterine adhesions	Epithelial cells of the oral mucosa	Using temperature-sensitive well plate inserts for culture plates and NIH-3T3 feeder cells.	[46]
Postoperative reconstruction of the middle ear mucosa	Epithelial cells of the nasal mucosa	Using temperature-sensitive well plate inserts for culture plates.	[47]

(“active” method) or by submerged adsorption (“passive” method). It was found that microgels actively deposited on the surface have smaller imprints and are more densely packed, compared to the reduced surface spread of globular proteins at strong deposition from the flow. Using this advantage, “active” deposition was demonstrated for the production of polyelectrolyte multilayer materials containing anionic microgels and a cationic linear polymer. Such multilayer microgels have been shown to effectively block the underlying substrate for the adhesion of macrophages, which is interesting, for example, for the modulation of the inflammatory response to implanted biomaterials.

Additionally, Wang et al. studied the use of self-assembling microgels for the suppression of bacterial colonisation of synthetic surfaces [54]. In this case, two antimicrobial mechanisms were investigated, namely: 1) modulation of cell surface adhesion; and 2) local storage/release of antimicrobial substances. For this, PEG-based microgels and a copolymer of PEG with acrylic acid (PEG-AA) were synthesized by

the suspension photopolymerisation method, and the obtained microgels were deposited on silicon coated with poly-L-lysine, forming a submonolayer coating. After deposition, a cationic antimicrobial peptide (L5) was introduced into the microgel, and the peptide content was significantly higher in PEG-AA microgels than in pure PEG microgels due to the electrostatic factor. Coating a peptide-free silicon substrate with a PEG-AA microgel significantly reduced surface colonisation by *S. epidermidis* and the degree of inhibition increased with the decrease in the average distance between microgels and surface bonds. The introduction of an L5 peptide into microgels after deposition further reduced coating colonisation with *S. epidermidis* to the low value observed for the control macroscopic PEG gel.

Wang and Libera investigated the surface deposition of microgels formed by the suspension polymerisation of AA and PEG on silicon surfaces modified with polylysine and the effect of their granular nature on protein adsorption [55]. Surface-bound PEG-AA microgels effectively

prevented the adsorption of fibronectin. In contrast, unprotected polylysine between adhered microgel particles (subconfluent coating) readily adsorbed this protein, thereby creating a disordered array of submicron-sized non-adhesive regions on the adherent cell surface. It has been found that compared to completely adhered surfaces, microgel coatings lead to rapid distribution and proliferation of cells, while the direction of differentiation does not change. Scanning electron microscopy (SEM) studies demonstrated that osteoblasts grow above the surface of the microgel, adhering to the surface areas exposed to polylysine, while optical microscopy with temporal resolution demonstrates higher cell mobility on the surface covered with microgel. These findings correspond with numerous studies of biomaterial surfaces structured in different ways and are consistent with the concept of intercellular interactions on the surface, regulated by the spatial distribution of cell adhesion sites. In addition, they suggest that microgel adsorption may represent an interesting way to control cellular processes involved in healing after biomaterial implantation.

Similar *Tsai et al.* investigated PNIPAAm microgels deposited on polystyrene substrates by immersion [56]. As the substrate removal rates were changed, surface structures were formed. Stripes of densely packed PNIPAAm microgels separated by intervals containing sparsely distributed microgels were clearly visible on these surface structures. It was found that NIH-3T3 cells plated on such micropatterned substrates, are fixed predominantly in the spaces, forming cell agglomerates. The cells formed confluent cell layers three days after plating. The extraction of fibroblast cell layers from substrates was carried out by lowering the temperature due to the temperature-sensitivity of the underlying layer of PNIPAAm microgel, similar to other modified PNIPAAm layers or differently reacting surfaces for collecting cell layers.

Considering the problem of interaction of cells with various surface features of granular microgel films, *Lynch et al.* studied the production of polymer coatings with controlled surface topography on a micrometre scale using microgel particles [57]. By changing the interaction between the microgel particles, the particles

were phase-separated into dense and particle-depleted domains, which remained on the surface after evaporation of the solvent. When the particle size changes, the size of the formed pores and their distribution in the film also change. It was shown that such systems can be formed into various structures, even obtained from microgel particles of the same size and the same composition. For *HeLa* cells grown on the surfaces of microgel based on 200 nm tert-butylacrylamide/isopropylacrylamide, cells could either grow in the pores of the microgel, where their distribution was limited by the pore size, or they could grow along dense domains between the pores, in this case, the cells had an elongated shape.

Li et al. focused on controlled surface heterogeneity, combining the assembly with control of surface wettability by microdroplets using embossing with polydimethylsiloxane and subsequent fixation for the assemblage of thousands of heterogeneous three-dimensional microenvironments for cells with precise control of individual shapes, sizes, chemical concentrations, cell density, and three-dimensional spatial distribution of many components [58].

The biological reactions on surfaces coated with a microgel have been most fully described in the studies of *Bridges et al.* devoted to the biological response of thin films formed by PNIPAAm microgels crosslinked with polyethylene glycol diacrylate [59]. These particles were grafted onto a conformally coated PET substrate, which was found to significantly reduce fibrinogen adsorption as well as adhesion and proliferation of primary human monocytes/macrophages. It was also found that microgel coatings lead to a decrease in the adhesion of leukocytes, as well as anti-inflammatory cytokines (TNF α , IL-1 β , MCP-1) after intraperitoneal implantation.

Evaluating the biological response of a microgel-coated surface *in vivo*, *Bridges et al.* investigated chronic inflammatory reactions to microgel coatings consisting of PNIPAAm microparticles crosslinked with polyethylene glycol diacrylate applied on PET [60]. At the same time, unmodified and microgel-coated PET discs were implanted subcutaneously in rats for 4 weeks, and the explants were analysed using histology and immunohistochemistry. Microgel

coatings have been found to reduce chronic inflammation and result in thinner fibrous capsules that contain 40% less cells compared to unmodified PET discs. In addition, the microgel coated samples contained significantly higher levels of macrophages (80%) than unmodified PET. These results demonstrate that microgel coatings reduce chronic inflammation caused by implanted biomaterials.

However, there are also reports that microgel coatings do not provide improved performance and biological response to biomaterials. Thus, considering that the effectiveness of neural electrodes implanted in the brain is often limited by host responses in the surrounding brain tissue, including astrocytic scar formation, neuronal cell death, and inflammation. *Gutowski et al.* investigated the host response to silicon neural electrodes with surface coatings formed by PNIPAAm-co-acrylic acid-PEG microgels and without them [61]. The adhesion of astrocytes and microglia for microgel-coated electrodes compared to uncoated controls was significantly reduced *in vitro*. In addition, the microgel coatings reduced the array of astrocytes around the implant for electrodes implanted in a rat's cerebral cortex. However, the microglia response indicated persistent inflammation, and the density of neurons around the implanted electrodes was lower for both groups of implants in comparison with the intact sample. Thus, it was concluded that microgel coatings did not significantly improve host response to implanted neural electrodes.

Although the main focus in the context of biomaterials has been on implant-related issues, the use of microgel coatings offers great opportunities, for example, for cell differentiation and growth, which can be used either as coatings for the surface of biomaterials or for applications in regenerative medicine.

Similarly with surfaces modified by grafting or adsorption of PNIPAAm or other temperature-sensitive polymers [62], cell adhesion improves at elevated temperatures (e.g. when bonds are broken due to deterioration of dissolution conditions), while cell detachment occurs when the temperature decreases or the solubility of PNIPAAm chains increases, causing the swelling of chains. By examining these effects, *Schmidt*

and co-authors demonstrated that PNIPAAm microgel films can be used for the controlled separation of adsorbed cells using temperature-sensitivity [63]. In this case, the properties of the microgel in the adsorbed state, as well as their changes with a change in temperature, were studied using the atomic force microscopy (AFM) method. Analysis shows that water content, surface adhesion, and nanomechanical properties change dramatically when the polymer film reaches a critical temperature, thus creating the basis for the rapid response of cells to temperature changes, both in terms of the number of fixed cells and their morphology. Similar results were also presented by *Uhlig et al.* [64].

4. Conclusions

The study of pH- and temperature-sensitive copolymers for medical use is a very promising area. This conclusion can be made after the analysis of a significant number of studies devoted to the use of such materials both as containers for delivery of medicinal substances, and as functional biomaterials, using which it is possible to create coatings for implants, as well as an artificial matrix for cell and tissue regeneration. Such smart polymers allow the flexible adjustment of the properties required in each specific case under the conditions in which they will perform their functions as efficiently as possible. This can be done by varying the composition of the copolymer, the degree of crosslinking of macromolecules, the use of various stabilising and initiating systems, as well as different methods of synthesis. Thus, pH- and temperature-sensitive polymer and copolymer materials provide researchers with a powerful tool that can change the approach to the treatment of many serious diseases.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Gisser K. R. C., Geselbracht M. J., Cappellari A., Hunsberger L., Ellis A. B., Perepezko J., et al. Nickel-titanium memory metal: A «Smart» material exhibiting a solid-state phase change and superelasticity. *Journal*

- of *Chemical Education*. 1994;71(4): 334. DOI: <https://doi.org/10.1021/ed071p334>
2. Erman B., Flory P.J. Critical phenomena and transitions in swollen polymer networks and in linear macromolecules. *Macromolecules*. 1986;19(9): 2342–2353. DOI: <https://doi.org/10.1021/ma00163a003>
 3. Tanaka T., Fillmore D., Sun S.-T., Nishio I., Swislow G., Shah A. Phase transitions in ionic gels. *Physical Review Letters*. 1980;45(20): 1636–1639. DOI: <https://doi.org/10.1103/physrevlett.45.1636>
 4. *Polymer Gels*. DeRossi D., Kajiwara K., Osada Y., Yamauchi A. (eds.). Boston, MA: Springer US; 1991. 354 p. DOI: <https://doi.org/10.1007/978-1-4684-5892-3>
 5. Ilmain F., Tanaka T., Kokufuta E. Volume transition in a gel driven by hydrogen bonding. *Nature*. 1991;349(6308): 400–401. DOI: <https://doi.org/10.1038/349400a0>
 6. Kuhn W., Hargitay B., Katchalsky A., Eisenberg H. Reversible dilation and contraction by changing the state of ionization of high-polymer acid networks. *Nature*. 1950;165(4196): 514–516. DOI: <https://doi.org/10.1038/165514a0>
 7. Steinberg I. Z., Oplatka A., Katchalsky A. Mechanochemical engines. *Nature*. 1966;210(5036): 568–571. DOI: <https://doi.org/10.1038/210568a0>
 8. Tian H., Tang Z., Zhuang X., Chen X., Jing X. Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. *Progress in Polymer Science*. 2012;37(2): 237–280. DOI: <https://doi.org/10.1016/j.progpolymsci.2011.06.004>
 9. Gonçalves C., Pereira P., Gama M. Self-Assembled hydrogel nanoparticles for drug delivery applications. *Materials*. 2010;3(2): 1420–1460. DOI: <https://doi.org/10.3390/ma3021420>
 10. Pangburn T. O., Petersen M. A., Waybrant B., Adil M. M., Kokkoli E. Peptide- and Aptamer-functionalized nanovectors for targeted delivery of therapeutics. *Journal of Biomechanical Engineering*. 2009;131(7): 074005. DOI: <https://doi.org/10.1115/1.3160763>
 11. Caldorera-Moore M. E., Liechty W. B., Peppas N. A. Responsive theranostic systems: integration of diagnostic imaging agents and responsive controlled release drug delivery carriers. *Accounts of Chemical Research*. 2011;44(10): 1061–1070. DOI: <https://doi.org/10.1021/ar2001777>
 12. Das M., Sanson N., Fava D., Kumacheva E. Microgels loaded with gold nanorods: photothermally triggered volume transitions under physiological conditions†. *Langmuir*. 2007;23(1): 196–201. DOI: <https://doi.org/10.1021/la061596s>
 13. Oh J. K., Lee D. I., Park J. M. Biopolymer-based microgels/nanogels for drug delivery applications. *Progress in Polymer Science*. 2009;34(12): 1261–1282. DOI: <https://doi.org/10.1016/j.progpolymsci.2009.08.001>
 14. Oh J. K., Drumright R., Siegwart D. J., Matyjaszewski K. The development of microgels/nanogels for drug delivery applications. *Progress in Polymer Science*. 2008;33(4): 448–477. DOI: <https://doi.org/10.1016/j.progpolymsci.2008.01.002>
 15. Talelli M., Hennink W. E. Thermosensitive polymeric micelles for targeted drug delivery. *Nanomedicine*. 2011;6(7): 1245–1255. DOI: <https://doi.org/10.2217/nnm.11.91>
 16. Bromberg L., Temchenko M., Hatton T. A. Smart microgel studies. Polyelectrolyte and drug-absorbing properties of microgels from polyether-modified poly(acrylic acid). *Langmuir*. 2003;19(21): 8675–8684. DOI: <https://doi.org/10.1021/la030187i>
 17. Vinogradov S. V. Polymeric nanogel formulations of nucleoside analogs. *Expert Opinion on Drug Delivery*. 2007;4(1): 5–17. DOI: <https://doi.org/10.1517/17425247.4.1.5>
 18. Vinogradov S. V. Colloidal microgels in drug delivery applications. *Current Pharmaceutical Design*. 2006;12(36): 4703–4712. DOI: <https://doi.org/10.2174/138161206779026254>
 19. Kabanov A. V., Vinogradov S. V. Nanogels as pharmaceutical carriers: finite networks of infinite capabilities. *Angewandte Chemie International Edition*. 2009;48(30): 5418–5429. DOI: <https://doi.org/10.1002/anie.200900441>
 20. Lee E. S., Gao Z., Bae Y. H. Recent progress in tumor pH targeting nanotechnology. *Journal of Controlled Release*. 2008;132(3): 164–170. DOI: <https://doi.org/10.1016/j.jconrel.2008.05.003>
 21. Dong H., Mantha V., Matyjaszewski K. Thermally responsive PM(EO)2MA magnetic microgels via activators generated by electron transfer atom transfer radical polymerization in miniemulsion. *Chemistry of Materials*. 2009;21(17): 3965–3972. DOI: <https://doi.org/10.1021/cm901143e>
 22. Nayak S., Lyon L. A. Soft nanotechnology with soft nanoparticles. *Angewandte Chemie International Edition*. 2005;44(47): 7686–7708. DOI: <https://doi.org/10.1002/anie.200501321>
 23. Hennink W. E., van Nostrum C. F. Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews*. 2012;64: 223–236. DOI: <https://doi.org/10.1016/j.addr.2012.09.009>
 24. Motornov M., Roiter Y., Tokarev I., Minko S. Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. *Progress in Polymer Science*. 2010;35(1-2): 174–211. DOI: <https://doi.org/10.1016/j.progpolymsci.2009.10.004>
 25. Saunders B. R., Laajam N., Daly E., Teow S., Hu X., Stepto R. Microgels: From responsive polymer colloids to biomaterials. *Advances in Colloid and Interface Science*. 2009;147-148: 251–262. DOI: <https://doi.org/10.1016/j.cis.2008.08.008>
 26. Landfester K. Miniemulsion polymerization and the structure of polymer and hybrid nanoparticles.

chemInform. 2009;40(33). DOI: <https://doi.org/10.1002/chin.200933279>

27. Seo M., Nie Z., Xu S., Mok M., Lewis P.C., Graham R., et al. Continuous microfluidic reactors for polymer particles. *Langmuir*. 2005;21(25): 11614–11622. DOI: <https://doi.org/10.1021/la050519e>

28. Nie Z., Li W., Seo M., Xu S., Kumacheva E. Janus and ternary particles generated by microfluidic synthesis: design, synthesis, and self-assembly. *Journal of the American Chemical Society*. 2006;128(29): 9408–9412. DOI: <https://doi.org/10.1021/ja060882n>

29. Seiffert S., Thiele J., Abate A. R., Weitz D. A. Smart microgel capsules from macromolecular precursors. *Journal of the American Chemical Society*. 2010;132(18): 6606–6609. DOI: <https://doi.org/10.1021/ja102156h>

30. Rossow T., Heyman J. A., Ehrlicher A. J., Langhoff A., Weitz D. A., Haag R., et al. Controlled synthesis of cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics. *Journal of the American Chemical Society*. 2012;134(10): 4983–4989. DOI: <https://doi.org/10.1021/ja300460p>

31. Perry J. L., Herlihy K. P., Napier M. E., DeSimone J. M. PRINT: A novel platform toward shape and size specific nanoparticle theranostics. *Accounts of Chemical Research*. 2011;44(10): 990–998. DOI: <https://doi.org/10.1021/ar2000315>

32. Caruso F., Sukhorukov G. Coated Colloids: Preparation, characterization, assembly and utilization. In: Decher G., Schlenoff J. B., editors. *Multilayer Thin Films*. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA; 2002. 331–362 p.

33. Sauzedde F., Elaïssari A., Pichot C. Hydrophilic magnetic polymer latexes. 2. Encapsulation of adsorbed iron oxide nanoparticles. *Colloid & Polymer Science*. 1999;277(11): 1041–1050. DOI: <https://doi.org/10.1007/s003960050488>

34. Sauzedde F., Elaïssari A., Pichot C. Hydrophilic magnetic polymer latexes. 1. Adsorption of magnetic iron oxide nanoparticles onto various cationic latexes. *Colloid & Polymer Science*. 1999;277(9): 846–855. DOI: <https://doi.org/10.1007/s003960050461>

35. Pich A., Richtering W. Microgels by Precipitation Polymerization: Synthesis, Characterization, and Functionalization. In: Pich A., Richtering W. (eds.) *Chemical Design of Responsive Microgels*. Springer Heidelberg Dordrecht London New York; 2011. p. 1–37. DOI: <https://doi.org/10.1007/978-3-642-16379-1>

36. Yamada N., Okano T., Sakai H., Karikusa F., Sawasaki Y., Sakurai Y. Thermo-responsive polymeric surfaces; control of attachment and detachment of cultured cells. *Die Makromolekulare Chemie, Rapid Communications*. 1990;11(11): 571–576. DOI: <https://doi.org/10.1002/marc.1990.030111109>

37. Kushida A., Yamato M., Konno C., Kikuchi A., Sakurai Y., Okano T. Decrease in culture temperature releases monolayer endothelial cell sheets together

with deposited fibronectin matrix from temperature-responsive culture surfaces. *Journal of Biomedical Materials Research*. 1999;45(4): 355–362. DOI: [https://doi.org/10.1002/\(sici\)1097-4636\(19990615\)45:4<355::aid-jbm10>3.0.co;2-7](https://doi.org/10.1002/(sici)1097-4636(19990615)45:4<355::aid-jbm10>3.0.co;2-7)

38. Sekine H., Shimizu T., Dobashi I., Matsuura K., Hagiwara N., Takahashi M., et al. Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in comparison with dissociated cell injection. *Tissue Engineering Part A*. 2011;17(23-24): 2973–2980. DOI: <https://doi.org/10.1089/ten.tea.2010.0659>

39. Nishida K., Yamato M., Hayashida Y., Watanabe K., Yamamoto K., Adachi E., et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *The New England Journal of Medicine*. 2004;351(12): 1187–1196. DOI: <https://doi.org/10.1056/nejmoa040455>

40. Kanzaki M., Yamato M., Yang J., Sekine H., Kohno C., Takagi R., et al. Dynamic sealing of lung air leaks by the transplantation of tissue engineered cell sheets. *Biomaterials*. 2007;28(29): 4294–4302. DOI: <https://doi.org/10.1016/j.biomaterials.2007.06.009>

41. Iwata T., Yamato M., Tsuchioka H., Takagi R., Mukobata S., Washio K., et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials*. 2009;30(14): 2716–2723. DOI: <https://doi.org/10.1016/j.biomaterials.2009.01.032>

42. Sawa Y., Miyagawa S., Sakaguchi T., Fujita T., Matsuyama A., Saito A., et al. Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. *Surgery Today*. 2012;42(2): 181–184. DOI: <https://doi.org/10.1007/s00595-011-0106-4>

43. Ohki T., Yamato M., Ota M., Takagi R., Murakami D., Kondo M., et al. Prevention of esophageal stricture after endoscopic submucosal dissection using tissue-engineered cell sheets. *Gastroenterology*. 2012;143(3): 582–588. DOI: <https://doi.org/10.1053/j.gastro.2012.04.050>

44. Ebihara G., Sato M., Yamato M., Mitani G., Kutsuna T., Nagai T., et al. Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model. *Biomaterials*. 2012;33(15): 3846–3851. DOI: <https://doi.org/10.1016/j.biomaterials.2012.01.056>

45. Sato M., Yamato M., Hamahashi K., Okano T., Mochida J. Articular cartilage regeneration using cell sheet technology. *The Anatomical Record*. 2014;297(1): 36–43. DOI: <https://doi.org/10.1002/ar.22829>

46. Kuramoto G., Takagi S., Ishitani K., Shimizu T., Okano T., Matsui H. Preventive effect of oral mucosal epithelial cell sheets on intrauterine adhesions. *Human Reproduction*. 2014;30(2): 406–416. DOI: <https://doi.org/10.1093/humrep/deu326>

47. Yamamoto K., Yamato M., Morino T., Sugiyama H., Takagi R., Yaguchi Y., et al. Middle ear

- mucosal regeneration by tissue-engineered cell sheet transplantation. *NPJ Regenerative Medicine*. 2017;2(1): 6. DOI: <https://doi.org/10.1038/s41536-017-0010-7>
48. Gan D., Lyon L. A. Synthesis and Protein adsorption resistance of PEG-modified poly(N-isopropylacrylamide) core/shell microgels. *Macromolecules*. 2002;35(26): 9634–9639. DOI: <https://doi.org/10.1021/ma021186k>
49. Veronese F. M., Mero A. The impact of PEGylation on biological therapies. *BioDrugs*. 2008;22(5): 315–329. DOI: <https://doi.org/10.2165/00063030-200822050-00004>
50. Sahay G., Alakhova D. Y., Kabanov A. V. Endocytosis of nanomedicines. *Journal of Controlled Release*. 2010;145(3): 182–195. DOI: <https://doi.org/10.1016/j.jconrel.2010.01.036>
51. Nolan C. M., Reyes C. D., Debord J. D., García A. J., Lyon L. A. Phase transition behavior, protein adsorption, and cell adhesion resistance of poly(ethylene glycol) cross-linked microgel particles. *Biomacromolecules*. 2005;6(4): 2032–2039. DOI: <https://doi.org/10.1021/bm0500087>
52. Scott E. A., Nichols M. D., Cordova L. H., George B. J., Jun Y.-S., Elbert D. L. Protein adsorption and cell adhesion on nanoscale bioactive coatings formed from poly(ethylene glycol) and albumin microgels. *Biomaterials*. 2008;29(34): 4481–4493. DOI: <https://doi.org/10.1016/j.biomaterials.2008.08.003>
53. South A. B., Whitmire R. E., García A. J., Lyon L. A. Centrifugal deposition of microgels for the rapid assembly of nonfouling thin films. *ACS Applied Materials & Interfaces*. 2009;1(12): 2747–2754. DOI: <https://doi.org/10.1021/am9005435>
54. Wang Q., Uzunoglu E., Wu Y., Libera M. Self-assembled poly(ethylene glycol)-co-acrylic acid microgels to inhibit bacterial colonization of synthetic surfaces. *ACS Applied Materials & Interfaces*. 2012;4(5): 2498–2506. DOI: <https://doi.org/10.1021/am300197m>
55. Wang Q., Libera M. Microgel-modified surfaces enhance short-term osteoblast response. *Colloids and Surfaces B: Biointerfaces*. 2014;118: 202–209. DOI: <https://doi.org/10.1016/j.colsurfb.2014.04.002>
56. Tsai H.-Y., Vats K., Yates M. Z., Benoit D. S. W. Two-dimensional patterns of poly(N-isopropylacrylamide) microgels to spatially control fibroblast adhesion and temperature-responsive detachment. *Langmuir*. 2013;29(39): 12183–12193. DOI: <https://doi.org/10.1021/la400971g>
57. Lynch I., Miller I., Gallagher W. M., Dawson K. A. Novel method to prepare morphologically rich polymeric surfaces for biomedical applications via phase separation and arrest of microgel particles. *The Journal of Physical Chemistry B*. 2006;110(30): 14581–14589. DOI: <https://doi.org/10.1021/jp061166a>
58. Li Y., Chen P., Wang Y., Yan S., Feng X., Du W., et al. Rapid assembly of heterogeneous 3D cell microenvironments in a microgel array. *Advanced Materials*. 2016;28(18): 3543–3548. DOI: <https://doi.org/10.1002/adma.201600247>
59. Bridges A. W., Singh N., Burns K. L., Babensee J. E., Andrew Lyon L., García A. J. Reduced acute inflammatory responses to microgel conformal coatings. *Biomaterials*. 2008;29(35): 4605–4615. DOI: <https://doi.org/10.1016/j.biomaterials.2008.08.015>
60. Bridges A. W., Whitmire R. E., Singh N., Templeman K. L., Babensee J. E., Lyon L. A., et al. Chronic inflammatory responses to microgel-based implant coatings. *Journal of Biomedical Materials Research Part A*. 2010;94A(1): 252–258. DOI: <https://doi.org/10.1002/jbm.a.32669>
61. Gutowski S. M., Templeman K. L., South A. B., Gauding J. C., Shoemaker J. T., LaPlaca M. C., et al. Host response to microgel coatings on neural electrodes implanted in the brain. *Journal of Biomedical Materials Research Part A*. 2014;102(5): 1486–1499. DOI: <https://doi.org/10.1002/jbm.a.34799>
62. da Silva R. M. P., Mano J. F., Reis R. L. Smart thermoresponsive coatings and surfaces for tissue engineering: switching cell-material boundaries. *Trends in Biotechnology*. 2007;25(12): 577–583. DOI: <https://doi.org/10.1016/j.tibtech.2007.08.014>
63. Schmidt S., Zeiser M., Hellweg T., Duschl C., Fery A., Möhwald H. Adhesion and mechanical properties of PNIPAM microgel films and their potential use as switchable cell culture substrates. *Advanced Functional Materials*. 2010;20(19): 3235–3243. DOI: <https://doi.org/10.1002/adfm.201000730>
64. Uhlig K., Wegener T., He J., Zeiser M., Bookhold J., Dewald I., et al. Patterned thermoresponsive microgel coatings for noninvasive processing of adherent cells. *Biomacromolecules*. 2016;17(3): 1110–1116. DOI: <https://doi.org/10.1021/acs.biomac.5b01728>

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