Smart Hydrogels in Lab-on-a-Chip (LOC) Applications

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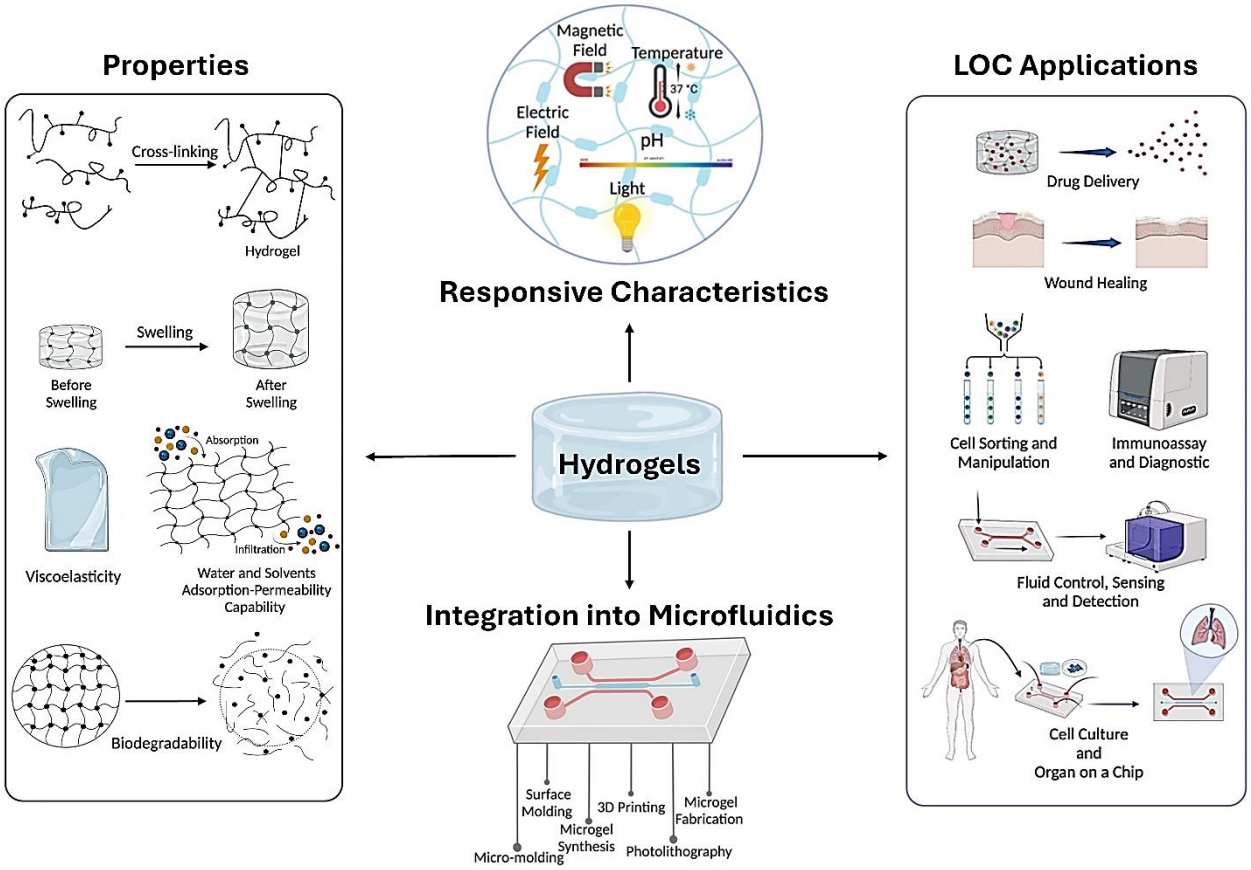
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Graphical Abstract



Abstract

Laboratory on-chip (LOC) technology facilitates numerous developments across diverse disciplines, such as medicine, tissue engineering, materials science, biomedical engineering, and biotechnology. Moreover, the potential applications appear boundless when LOC is integrated with intelligent hydrogels. In the literature, however, there are few accounts of the vast array of developments and applications that this combination has spawned. These new systems, which integrate smart hydrogels and LOC and thus significantly advance cutting-edge technology, have been thoroughly examined in this review. The functions of smart hydrogels in LOC applications were described and subsequently the developed intelligent hydrogels were classified as multi-responsive, thermo-responsive, pH-responsive, and stimuli-responsive (light, magnetic, and electric). Following this, details regarding tunable properties for LOC functions were provided, followed by a discussion of the fabrication processes and integration of these intelligent hydrogels into LOC systems, including their benefits and drawbacks. Following that, current literature examples of LOC systems utilizing these intelligent hydrogels for biosensing, 3D culture, tissue engineering, controlled release, personalized medicine, drug delivery, analyte enrichment, and organ-on-a-chip applications were presented. Following the presentation of state-of-the-art information regarding smart hydrogel characterization techniques, present challenges and prospective prospects were discussed.

Keywords: hydrogels, lab-on-a-chip, responsive materials.

Introduction

Laboratory on-chip (LOC) technology is a revolutionary approach to minimizing and automating laboratory processes, as it enables the integration of multiple analytical functions into a single micro liquid device [1]. LOC technology, also known as micro-fluid systems, is a multidisciplinary discipline that combines biology, chemistry, physics, and engineering to create miniature analytical devices capable of performing a variety of laboratory functions [2]. In the last few decades, the laboratory-on-a-chip concept has attracted significant interest due to its potential to revolutionize the way we conduct experiments and make diagnoses [3]. The origins of LOC technology can be traced back to the 1970s, when microfabrication techniques were first used to create miniature devices for analytical purposes, but before the 1990s the term "lab on chip" was not widely used [4]. Microelectronics, microfluidics, and material science advancements have had a significant impact on the development of LOC technology. Considerable milestones in this field include the invention of microfluidic valves, pumps, and channels, as well as the integration of sensors and detectors into microfluidic devices, and these developments form the basis of today's modern LOC systems [5].

Lab-on-a-chip devices typically have central micro-channels through which liquids are manipulated at the microliter or even nanoliter scale, allowing transportation, mixing, and separation of samples and reagents [6]. Typically, micro-valves and micro-pumps are incorporated into LOC devices to regulate precise fluid flow within these micro-channels and automate processes. Moreover, sensors and detectors are frequently applied to LOC devices to perform analytical measurements and are generally preferred for collecting optical, electrochemical and thermal data [7,8]. LOC technology is based on the ability to perform multiple laboratory functions, such as sampling, combining, reaction, and detection, on a compact platform and they are fabricated using microfabrication techniques such as photolithography, soft lithography, and micro processing [9]. These methods allow precise creation of micro-channels and other components on a chip, and this inclusive integrated system provides benefits such as reduced sample and reagent volumes, shorter reaction times, and increased portability [10,11].

Hydrogels are stable, three-dimensional networks of crosslinked polymeric molecules that can absorb vast quantities of water or biological fluids. Hydrophilic functional groups like -NH_2, -OH, -COOH, and -SO_3H in the polymer provide hydrogels their water-adsorption capability,

whereas crosslinks between network chains give them their resistance to disintegration. Hydrogels are known to absorb a diverse spectrum of solvents, not limited to water. Hydrogels also allow a lot of oxygen, nutrients, and other metabolites that dissolve in water to flow through [12,13].

Hydrogels' versatile nature and compatibility with biological systems make them valuable tools in the field of biomedicine [14]. Hydrogels can be tailored to allow small molecule permeability, crucial for drug delivery systems and their optical transparency is advantageous for applications like imaging and diagnostics. The use of hydrogels in biomedicine aligns with the growing demand for green-technology-based materials, as they can be derived from biopolymers like polysaccharides, enhancing their biocompatibility [15]. The history of hydrogels in LOC systems began with their use in providing a hydrated and biocompatible environment. Initially, hydrogels were valued for their mechanical properties and ability to mimic natural tissue structures [16]. As microfluidic systems advanced, hydrogels became integral in enhancing the functionality of these devices. The introduction of smart hydrogels marked a significant evolution, with their ability to respond to stimuli such as temperature, pH, and light [17]. This responsiveness allows for precise control and dynamic adjustments within LOC systems, making smart hydrogels crucial for the development of advanced and versatile LOC applications. The transition from basic hydrogels to smart hydrogels underscores their vital role in the ongoing innovation and enhancement of LOC technology [18]. In the realm of microfluidic devices, hydrogels are favored due to their ability to facilitate unrestricted diffusion, enhance fluid resistance, and establish an environment conducive to such processes. Sample preparation and analysis, cell encapsulation and culture, fluid control, sensing and detection, drug delivery systems, immunoassays and diagnostics, on-chip reagent storage, and cell sorting and manipulation are just a few of the functions performed by hydrogels in microfluidic chip applications [19].

Hydrogels have the capacity to function as a multifunctional separation matrix, which grants them the potential for seamless integration into microfluidic devices [20]. Electrophoresis, the preeminent method in this domain, has demonstrated exceptional efficacy and consequently emerged as a prominent application in a multitude of scientific fields [21]. The process of separating target analytes from a complex mixture can be accomplished by incorporating ligands or molecules strategically into the hydrogel matrix [22]. This methodology exhibits a

wide range of applications, including but not limited to high-throughput DNA decoding [23], comprehensive protein characterization [24], and pharmaceutical compound evaluation [25].

Hydrogels, owing to their biocompatible nature and ability to emulate the extracellular matrix, exhibit remarkable potential for encapsulating cells within microfluidic devices [26]. The utilization of this technique enables the establishment of controlled and meticulous cell culture systems, thereby facilitating the creation of microenvironments that are conducive to the study of cellular behavior, drug testing, and tissue engineering endeavors [27]. In recent years, the integration of hydrogel-based 3D cell culture systems within microfluidic chips has emerged as a promising approach in the field of biomedical research by offering a unique platform that holds great potential for generating more physiologically relevant results [28]. By encapsulating cells within hydrogel matrices, researchers are able to mimic the complex three-dimensional architecture and dynamic microenvironment of living tissues [29]. This enables the recreation of intricate cell-cell and cell-matrix interactions, which are crucial for understanding various biological processes and disease mechanisms [29]. Moreover, the incorporation of microfluidic channels inside these chips allows for precise control over the delivery of nutrients, oxygen, and other biochemical cues [30].

Hydrogels have also demonstrated remarkable potential as functional components within microfluidic chips, effectively assuming the roles of valves or pumps [31]. The regulation of fluid flow can be achieved through the utilization of hydrogels, which possess the remarkable ability to swell and de-swell in response to variations in environmental conditions such as pH or temperature [32]. This unique property enables hydrogels to effectively modulate fluid dynamics. The precise manipulation of samples and reagents within micro-channels is of utmost importance in various scientific and technological applications.

Hydrogels possess the remarkable capability of being endowed with specific molecules or nanoparticles, thereby assuming the role of sensing and/or detection elements within the realm of microfluidic devices [32]. The inherent adaptability of these entities enables them to undergo modifications in direct response to specific target analytes, thereby facilitating their subsequent detection and quantification [33]. The significance of this phenomenon cannot be overstated, particularly in its implications for the development and utilization of biosensors and environmental monitoring systems [34].

The utilization of hydrogels in the development of controlled drug delivery systems within microfluidic chips has garnered significant attention in recent years [35]. The precise control of release rate and dosage can be achieved by integrating drugs or therapeutic agents within the hydrogel matrix [36]. The utilization of this approach proves to be highly advantageous in the investigation of drug responses and the advancement of precise drug delivery systems.

In the realm of immunoassay applications, hydrogels have emerged as a promising platform for the capture and detection of specific biomarkers through the strategic modification with antibodies or antigens [37]. The utilization of microfluidic chips integrated with hydrogel-based capture assays holds immense value in the field of diagnostics and point-of-care testing [38]. This innovative approach enables the swift and highly sensitive detection of various diseases, making it an indispensable tool in healthcare settings.

In the field of microfluidic technology, hydrogels have emerged as a promising medium for the encapsulation and storage of reagents or samples [39]. This versatile class of materials, composed of a three-dimensional network of hydrophilic polymers, exhibits a remarkable capacity to retain aqueous solutions within their structure [40]. Leveraging this unique property, hydrogels have found widespread application in the realm of microfluidic chips, enabling the efficient containment and controlled release of various substances [41]. By harnessing the inherent properties of hydrogels, researchers have unlocked new avenues for manipulation and preservation, and the ability to release these desired substances at will not only simplifies the sample processing process but also reduces the risk of potential contamination, thus increasing the overall effectiveness of the experimental procedure [42]. The benefit of this technology is particularly evident in the field of portable diagnostic devices [43].

The use of hydrogels in microfluidic technology has emerged on the other hand as a promising approach for the capture, sorting, and manipulation of individual cells [43]. This new application has tremendous potential for scientific and biomedical studies and is of particular importance in terms of genomics and personalized medicine, such as single cell analysis, cell or tissue classification, characterization and personalized medicine [44,45].

In conclusion, hydrogels exhibit remarkable versatility when employed in microfluidic chip applications, showcasing an extensive repertoire of functionalities encompassing sample separation and analysis, cell culture, fluid manipulation, sensing capabilities, and drug administration. The wide range of uses of hydrogels in microfluidic chip applications are shown in Figure 1. The intrinsic value of their biocompatibility, ability to be finely adjusted, and capacity to react to alterations in their surroundings render them indispensable constituents in the advancement of cutting-edge microfluidic systems across a multitude of disciplines, encompassing biology, medicine, chemistry, and environmental science [46].

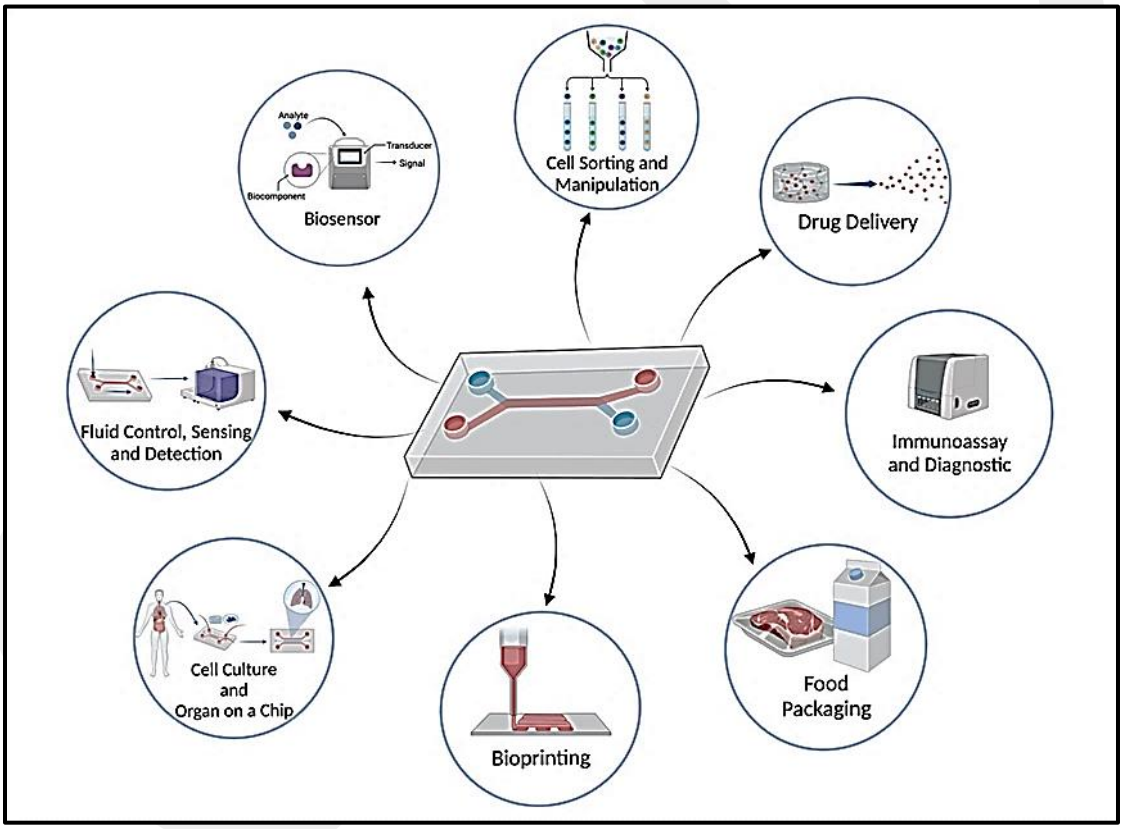


Figure 1. Summary of the uses of hydrogels in microfluidic chip applications.

1. Importance of Smart Hydrogels for Enhancing LOC Performance

As the demand for more efficient, accurate, and compact diagnostic and analytical devices grows, enhancing the performance of LOC systems becomes increasingly crucial [47]. LOC technologies, which integrate multiple laboratory functions onto a single chip, face challenges such as precise fluid control, rapid detection capabilities, and the ability to function in diverse environments [48]. Smart hydrogels, with their responsive properties, sensitivity, and selectivity, real-time monitoring and controllability, integration, and miniaturization, play a pivotal role in addressing these challenges by enhancing LOC performance [49].

Smart hydrogels can dynamically adjust their physical and chemical properties in response to environmental stimuli such as temperature, pH, ionic strength, and light. This adaptability is particularly valuable in LOC systems [50]. For instance, temperature-sensitive hydrogels like poly(N-isopropylacrylamide) (PNIPAAm) change their phase in response to temperature variations, allowing for controlled fluid flow and reagent release [51]. Similarly, pH-sensitive hydrogels can regulate fluid flow and membrane permeability within LOC devices, optimizing assay conditions for biochemical reactions [52].

The sensitivity and selectivity of LOC systems are significantly enhanced by smart hydrogels through tailored interactions with specific analytes. These hydrogels can be functionalized with molecular recognition elements such as antibodies, aptamers, or enzymes, which bind selectively to target molecules [53]. For example, enzyme-functionalized hydrogels have been employed in glucose sensors, where the hydrogel's enzymatic activity allows for precise glucose measurements in clinical diagnostics [54]. Another example includes aptamer-functionalized hydrogels used in detecting specific proteins or pathogens, enhancing the selectivity and sensitivity of the detection process, and reducing false positives [55].

Real-time monitoring and controllability are essential for many LOC applications, particularly those requiring continuous or adaptive responses to changing conditions. Smart hydrogels enable these capabilities by responding to dynamic stimuli in situ [56]. For instance, light-sensitive hydrogels used in optical sensors can alter their properties in response to light variations, enabling continuous measurement and real-time feedback. This capability is crucial for applications like point-of-care diagnostics, where the ability to adapt to fluctuating conditions ensures accurate and timely results [57]. A practical example is the use of

photopolymerizable hydrogels in creating tunable microenvironments for cell culture and analysis, providing real-time control over experimental conditions [58].

The integration and miniaturization capabilities of smart hydrogels support the development of compact, multifunctional LOC devices [59]. These hydrogels can be incorporated into various microfluidic components such as valves, pumps, and reactors, facilitating sophisticated fluid handling and processing within a small footprint [60]. For example, hydrogels with tunable swelling properties are used to create microvalves that precisely control fluid flow, essential for conducting multiple assays simultaneously on a single chip [61]. In another example, smart hydrogels have been integrated into LOC systems to create miniaturized platforms for high-throughput screening and analysis, reducing reagent consumption and increasing efficiency [62].

In conclusion, smart hydrogels significantly enhance the performance of LOC systems through their responsive properties, sensitivity and selectivity, real-time monitoring and controllability, and integration and miniaturization capabilities. By enabling precise control over microenvironments, supporting multifunctional device development, and providing continuous feedback, smart hydrogels advance the functionality and versatility of LOC technologies. As research progresses, the potential applications of smart hydrogels in LOC technology continue to expand, promising further innovations in biomedical diagnostics, environmental monitoring, and beyond.

2. Types and Characteristics of Smart Hydrogels

Smart hydrogels are diverse materials known for their ability to respond to various stimuli, making them invaluable in a wide range of applications. These include thermoresponsive, pH-responsive, stimuli-responsive (such as light and electric fields), and multi-responsive hydrogels, each tailored to exhibit specific behaviors based on environmental conditions. The characteristics of each type are summarized in Figure 2.

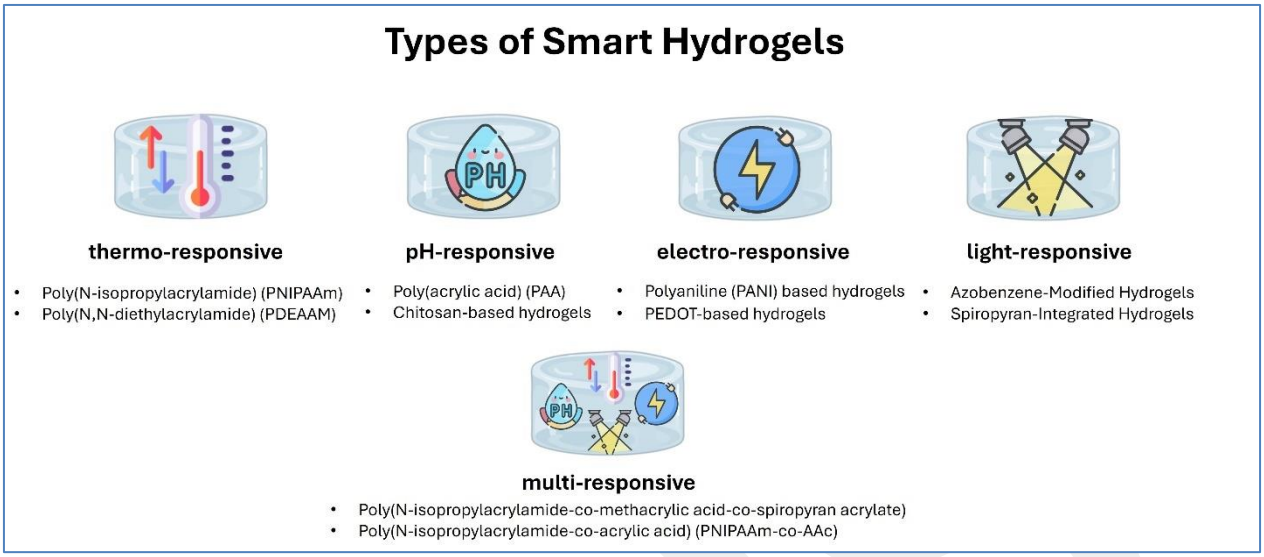


Figure 2. Types and widely known examples of smart hydrogels.

2.1. Thermo-responsive hydrogels

Thermo-responsive hydrogels, a class of smart materials, exhibit a remarkable ability to undergo a reversible phase transition upon exposure to variations in temperature [63]. The hydrogels under investigation manifest distinctive attributes, notably volume phase transitions, which have garnered considerable attention due to their remarkable sensitivity to external stimuli [64]. The hallmark feature exhibited by thermo-responsive hydrogels lies in their remarkable capacity to undergo a transition in their physical state contingent upon variations in temperature [64]. These hydrogels exhibit a remarkable transition in their physicochemical properties upon reaching a distinct temperature threshold, commonly referred to as the lower critical solution temperature (LCST). Below this critical temperature, these hydrogels demonstrate a pronounced affinity for water molecules, displaying a hydrophilic nature and exhibiting significant swelling behavior [65]. However, surpassing the LCST triggers a notable transformation, rendering the hydrogels hydrophobic in nature, leading to a collapse in their structural integrity [66]. The observed transition is a consequence of the dynamic interplay between hydrophilic and hydrophobic interactions within the intricate polymer network. One of the most extensively investigated instances in the field of stimuli-responsive polymers is poly(*N*-isopropylacrylamide) (PNIPAM), which exhibits an LCST of approximately 32°C [67]. In addition to PNIPAM, a variety of thermo-responsive hydrogels have been extensively

studied in the field including poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) (PEO-PPO-PEO) [68], poly(2-isopropyl-2-oxazoline) (PIPOZ) [69], poly(*N*-vinylcaprolactam) (PNVCL) [70], poly(ethyleneimine) (PEI) [71], Poly(acrylic acid) (PAA) [72], Poly(2-(2-methoxyethoxy)ethyl methacrylate) ($PMEO_2MA$) [73], and chitosan-*g*-poly(*N*-isopropylacrylamide) (CS-*g*-PNIPAM) [74]. These hydrogels have attracted significant attention due to their unique thermo-responsive properties and potential applications in various fields. In addition to the aforementioned examples, it is worth noting that the scientific literature offers further instances of notable polymers, including (-*g*-pNIPAM), poly(vinyl alcohol) (PVA), poly(ethylene glycol) [75–77].

2.2. pH-responsive Hydrogels

pH-responsive hydrogels represent a remarkable category of intelligent hydrogels, exhibiting the ability to undergo reversible alterations in their properties upon encountering variations in the pH levels of their ambient milieu [78]. The hydrogels under consideration have been meticulously engineered to exhibit a remarkable ability to undergo volumetric expansion or contraction in response to variations in the pH levels of their immediate environment [79]. pH-responsive hydrogels are commonly endowed with acidic or basic functional groups, such as carboxylic acid (-COOH) or amino ($-NH_2$) moieties, which enable them to exhibit remarkable responsiveness to changes in pH levels. The phenomenon of ionization or deionization exhibited by these groups in response to variations in pH serves as the driving force behind the volumetric expansion or contraction of the hydrogel material [80]. pH-responsive hydrogels exhibit a remarkable capacity to be meticulously tailored to elicit a targeted response within a specific pH spectrum, thereby endowing them with unparalleled adaptability for a myriad of diverse applications [81]. In physiological and biological contexts characterized by significant pH fluctuations, these entities have proven to be of extraordinary value, and this pH-dependent behavior is of highly significant value for a wide range of applications, including drug delivery systems, biosensors, and controlled release devices [82]. Numerous pH-responsive hydrogels have been identified as possessing an inherent biocompatibility, rendering them highly amenable for deployment in a diverse array of biomedical contexts [81]. Of particular significance is their potential utility in facilitating the controlled release of therapeutic agents within the intricate milieu of the human organism [83]. In the field of pH-responsive hydrogels, several notable examples emerge as recurrent subjects of investigation. Among these, poly(acrylic acid) (PAA) [84], poly(methacrylic acid) (PMAA) [85], chitosan [85], hydroxyethyl

methacrylate (HEMA) [86], polyvinylpyrrolidone (PVP) [87], poly(acrylamide-*co*-acrylic acid) (P(AAm-*co*-AA)) [88], alginates [89], and hydroxypropyl cellulose (HPC) [90] have garnered significant attention due to their distinct properties and characteristics.

2.3. Stimuli-responsive Hydrogels (light, magnetic, electric)

Stimuli-responsive hydrogels belong to a category of intelligent hydrogels capable of undergoing reversible alterations in their physical properties when exposed to external stimuli [91]. The stimuli encompass various factors, such as light, magnetic fields, and electric fields, which enable the remote manipulation of processes like cell alignment or drug release, among others [91]. Light-responsive hydrogels undergo a transformation in their characteristics upon exposure to particular wavelengths of light [92]. This may encompass alterations in swelling, stiffness, or the liberation of encapsulated substances. Magnetic-responsive hydrogels are composed of magnetic nanoparticles or materials that exhibit a responsive behavior when subjected to external magnetic fields [93]. The hydrogel has the ability to undergo changes in shape, orientation, or properties when subjected to the influence of a magnetic field. Electric-responsive hydrogels exhibit a responsive behavior in the presence of electric fields [94]. Variations in voltage or current have the potential to modify the properties of the hydrogel, including its swelling behavior, mechanical strength, and conductivity.

In the category of light-responsive hydrogels, also referred to as photoresponsive hydrogels, several notable examples emerge, each distinguished by their unique mechanisms of light-induced response. Among these, we encounter the azobenzene-based hydrogels, which exhibit a remarkable capacity for light-driven transformations [95]. Similarly, spiropyran-based hydrogels captivate with their ability to undergo reversible photoisomerization upon exposure to light [95]. Additionally, the *o*-nitrobenzyl-based hydrogels manifest a pronounced sensitivity to light, leading to controlled release of encapsulated substances [96]. Lastly, the phthalocyanine-based hydrogels, with their distinctive molecular structure, exhibit intriguing light-responsive behavior [97]. These exemplars collectively represent a diverse array of light-responsive hydrogels, each offering unique avenues for scientific exploration and potential applications. On the other hand, there are various types of hydrogels that demonstrate magnetic responsiveness. These include hydrogels that are infused with magnetite (Fe_3O_4) or hematite (α-Fe_2O_3) nanoparticles, hydrogels that contain iron oxide (Fe_2O_3) nanoparticles, ferrogels, and hydrogels that incorporate magnetic nanocomposites [98]. Electrically

responsive hydrogels, commonly referred to as conductive hydrogels or electrosensitive hydrogels, encompass a range of materials including poly(3,4-ethylenedioxythiophene) (PEDOT)-based hydrogels [99], polyaniline (PANI)-based hydrogels [100], and polyacrylamide (PAAm) hydrogels [101] with conductive fillers such as carbon nanotubes [101] or graphene [101]. These hydrogels exhibit the ability to respond to electrical stimuli, making them highly attractive for various applications. On the other hand, notable instances include agarose-based hydrogels infused with conductive nanoparticles [101], as well as hydrogels derived from poly (2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) [102]. In addition, it is worth noting that there exist a class of hydrogels that exhibit electrical responsiveness, which are created through the integration of conductive materials into conventional hydrogel matrices [103,104]. The hydrogels discussed in this context are composite materials that consist of a combination of conductive polymers, nanoparticles, and hydrogel precursors [105].

2.4. Multi-responsive Hydrogels

Multi-responsive hydrogels are a class of materials that possess the ability to respond to various combinations of external stimuli such as pH, temperature, ionic strength, light exposure, magnetic fields, electrical signals, and molecular recognition elements [106]. The unique property of multi-responsiveness allows these hydrogels to be utilized in a diverse range of applications such as drug delivery, tissue engineering, bio sensing, and the development of responsive materials [106]. Examples of hydrogels that are sensitive to pH and temperature are found in the literature and these hydrogels are used to release drugs into the body at specific pH and temperature levels [107,108]. Due to their swelling and collapse behavior, pH and ionic strength sensitive hydrogels are also available and are used in tissue engineering applications as well as controlled drug release studies [109]. Photo-thermal therapy studies that react to temperature changes brought on by light exposure use hydrogels that are both temperature and light sensitive [92]. Certain hydrogels are engineered to react to variations in temperature, ionic strength, and pH [110]. Hydrogels with pH, temperature, and electrical signal sensitivity are more appropriate for tissue engineering and cell culture applications to produce dynamic microenvironments for cells [111]. Certain highly responsive hydrogels have molecular recognition components like aptamers or antibodies in addition to reacting to temperature, pH, and ionic strength and these types of hydrogels have applications in diagnostics and bio sensing [112].

3. Tunable properties of Hydrogels for Specific LOC functions

Hydrogels have emerged as highly versatile materials possessing the ability to exhibit tunable properties, rendering them amenable for customization to fulfill specific functions within the context of LOC devices [113]. The inherent tunability of these properties provides a high degree of precision in controlling and optimizing various functions within microfluidic systems, the most notable of which are permeability, biocompatibility, similarity with the ECM, transparency, inertness, responsiveness to environmental stimuli**,** controllable degradation rate, stiffness and swelling/deswelling ratio [114,115].

Permeability: The permeability of hydrogels is crucial in LOC applications due to its role in facilitating the generation of flexible gradient profiles, necessary for physiological and pathological behaviours such as angiogenesis and tumor migration [116]. The superior permeability of hydrogel materials allows for the creation of perfusable micro-channels with small cross-sections, enabling steady perfusion for extended periods [117]. However, it is essential to consider the response time of hydrogels, as it proportional to the square of the gel's characteristic dimension, which can impact their suitability for specific applications [118]. Additionally, the permeability of hydrogels affects vascular permeability with increased concentrations leading to elevated vascular permeability and increased extravasation [119]. Furthermore, the gas permeability of hydrogels is crucial for cell-based lab-on-a-chip devices, as insufficient oxygen supply can lead to hypoxia-induced reactions in cells [120].

Biocompatibility: The biocompatibility of hydrogels and similarity to ECM are of great importance for LOC applications as it enables the creation of suitable microenvironments for cell growth, tissue engineering, and organ-on-a-chip models [46]. Due to their high biocompatibility, they provide an ideal platform for cell adhesion and proliferation [121]. Additionally, the use of biocompatible hydrogels is essential for the fabrication of soft devices with robust actuating ability for soft robotics and microfluidic applications [122].

Transparency: The transparency of hydrogels is crucial for LOC applications as it influences optical detection, imaging, and visualization within microfluidic devices. Transparent hydrogels are essential for enabling real-time monitoring and visualization of cellular and molecular processes within microfluidic systems [123]. The optical transparency of hydrogels is vital for applications such as shear stress studies in neurovascular unit models, where brain

endothelial cells grown in optically transparent membranes withstand physiological fluid shear stress, allowing for direct visualization and analysis of cellular responses [124]. Additionally, the transparency of hydrogels is important for the development of microfluidic platforms for high-throughput single molecule/cell analysis, as it allows for the visualization and tracking of cellular behavior within the hydrogel matrix [125]. Moreover, the transparency of hydrogels is critical for the development of hydrogel based colorimetric assays for multiplexed microRNA detection in microfluidic devices, allowing for the visualization and quantification of micro-RNA expression levels, which is essential for disease diagnostics and point-of-care applications [126].

Inertness: In the context of LOC applications, the inertness of hydrogels ensures that they do not interfere with the biological or chemical components of the system, thereby preserving the integrity of the assays and experiments conducted within the microfluidic device [127]. This is particularly important for the long term cultivation of mammalian cells on chip, as it prevents undesirable interactions between the cells and the material [128]. Additionally, the inertness of hydrogels is essential for mimicking in vivo microenvironments, which is crucial for organ-on-a-chip systems and physiological studies [129].

Responsiveness to Environmental Stimuli: The responsiveness to environmental stimuli is crucial for hydrogels used in LOC applications due to their ability to adapt to surrounding environments and regulate the transport of ions and molecules, change wettability and adhesion of different species, and convert chemical and biochemical signals into optical, electric, thermal, and mechanical signals etc. [130]. This property is particularly important for the versatility of smart hydrogels, as it ensures instantaneous and remarkable feedback after receiving environmental signals and high extensibility and deformability to tolerate external forces [131]. Moreover, the responsiveness to multiple stimuli is of particular interest for DNA-based hydrogels due to their extremely specific molecular sensing functionalities, offering the possibility to combine DNA hydrogels with environmentally responsive polymers, enzymes, and functional nanomaterials to improve their applications [132].

Controllable Degradation Rate: The controllable degradation rate of hydrogels used in LOC applications is of great importance in terms of tailoring the stability, drug release kinetics, and tissue regeneration properties on the microfluidic devices, thereby enabling their effective use in various biomedical and diagnostic applications [133]. The ability to precisely regulate the

degradation rate of hydrogels essential for various biomedical applications, including bone tissue engineering, where the degradation rate should ideally match the rate of new tissue formation to provide adequate support for bone regeneration [134]. Additionally, the controlled degradation of hydrogels is essential for the sustained release of growth factors and drugs, which is critical for therapeutic strategies requiring specific durations of action [135]. Lastly, the tunable degradation rates of hydrogels can be achieved through careful selection of network chemistries, degradation kinetics, and network connectivity, which influence crosslink density and mass loss and this tenability allows for the customization of hydrogels to match specific application requirements [136].

Stiffness and Mechanical Properties: The stiffness is another key parameter of hydrogels used in LOC applications due to its significant influence on cell behavior, mechanotransduction, and the mechanical properties of the microfluidic devices [137]. Hydrogel stiffness plays a pivotal role in regulating cell spreading, differentiation, and response to mechanical cues within the microenvironment [138]. The cells encapsulated in hydrogels of varying stiffness exhibit different behaviors, impacting their morphology, adhesion, and signaling pathways [138]. Furthermore, the stiffness of the extracellular matrix is known to influence cell phenotype, inspiring work toward unraveling the role of stiffness on cell behavior using hydrogels [139]. Moreover, hydrogel stiffness is critical as it can influence the performance of on-chip components such as valves and waveguides [140]. Lastly, the stiffness of hydrogels may affect the optical and mechanical properties of waveguides, making them suitable for chemical sensing and optical applications within microfluidic devices [141].

Swelling/Deswelling Behavior: The swelling/deswelling behavior of the hydrogels allow absorb and release fluids, making them ideal for applications such as drug delivery and bio sensing [142]. Additionally, the mechanical strength and rigidity of hydrogels play a crucial role in maintaining the structural integrity of LOC devices [143]. Hydrogels also exhibit sensitivity to environmental stimuli, enabling them to respond to changes in temperature, pH, or light as mentioned in the above sections. This responsiveness can be harnessed for various purposes, including controlled release of drugs or triggered actuation of microfluidic components. The functionalization of hydrogels with biomolecules further enhances their versatility in LOC applications [144]. By incorporating specific biomolecules, such as antibodies or enzymes, hydrogels can be tailored to perform targeted sensing or bioactive functions. The porosity and pore size of hydrogels are critical parameters that influence their diffusion properties and cell

compatibility allowing precisely engineered to facilitate the transport of molecules or cells within LOC devices [145]. The degradability is another important property of hydrogels, particularly for applications requiring temporary or bio resorbable materials and the hydrogels with controlled degradation rates employed mostly for tissue engineering or implantable devices [136]. Finally, multi-responsiveness is a unique property of certain hydrogels, allowing them to simultaneously exhibit complex and dynamic control by responding to multiple stimuli over the LOC [146]. The ability of hydrogels to undergo reversible swelling and deswelling in response to external stimuli such as changes in pH, ionic strength, or temperature is essential for the development of smart microfluidic devices by assuring the precise control of fluid flow, analyte capture and release, and the modulation of microenvironmental conditions within the chip [147,148]. On the other hand, the reversible swelling and deswelling characteristics of hydrogels are essential for the development of stimuli responsive actuators, pumps and micro-valves, enabling dynamic and programmable fluidic operations within the chip [149].

In summary, the unique and adaptable properties of hydrogels enable their customization for both single-use and multiple-use applications within LOC systems. This adaptability not only enhances the stability and durability of these devices but also promotes cost-efficiency and sustainability. By precisely tailoring the characteristics of hydrogels, researchers and engineers are able to optimize device performance and extend their lifespan, effectively reducing both the economic and environmental burdens associated with their production and utilization. Ultimately, the tunable properties of hydrogels offer extensive possibilities for innovation in microfluidic systems across diverse sectors, including diagnostics, drug discovery, and environmental monitoring, underscoring their pivotal role in advancing current and future technological applications.

4. Micro-structuring of Smart Hydrogels for Adapting to LOC Devices

The process of hydrogel micro-structuring encompasses a variety of techniques for generating detailed microscale features inside hydrogel matrices. These techniques are essential for applications that include tissue engineering, drug delivery, and microfluidic devices [150]. These techniques are categorized as photolithography, microfluidics, 3D Printing, electrospinning, microgel synthesis, template-based approaches, solvent casting and particulate leaching, freeze casting and are illustrated in Figure 3.

Photolithography is a technique that is widely used to define detailed patterns. This technique makes use of photo-responsive hydrogels and ultraviolet light. Through the use of elastomeric stamps, surface micro-structuring can be made easier through the development of soft lithography, which incorporates micro-contact printing and replica molding [151]. When it comes to hydrogel patterning in limited channels, microfluidics is a reliable method that makes use of precise fluid manipulation. This allows for the formation of regulated microenvironments [152]. Layer-by-layer assembly of hydrogel structures is made possible by 3D printing technologies such as stereo-lithography and extrusion-based approaches. This allows for the creation of three-dimensional architectures that are specifically customized to the user's needs [153]. The technology of electrospinning has emerged as a versatile method that utilizes electric fields to create microfibers within hydrogels. These microfibers are useful for imitating the extracellular matrix material [154]. On the other hand, micro-gel synthesis is a crucial aspect of hydrogel micro-structuring, offering a versatile and tunable approach to create finely controlled structures. Micro-gels, which are micron-sized hydrogel particles, are typically synthesized through methods such as emulsion polymerization or precipitation polymerization. Micro-gels can then be incorporated into hydrogel matrices to impart specific functionalities or act as building blocks for complex structures [155].

The production of the hydrogel microstructure can also be guided by template-based approaches, which may include sacrificial or detachable templates. This ensures that the design is regulated and that the microstructure is flexible [156]. In the process of solvent casting and particulate leaching, hydrogels are mixed with particulate materials, and then the mixture is subjected to solvent casting and particle removal. This process ultimately results in the formation of porous micro-structured hydrogels [157]. Lastly, the freeze casting is a versatile technique employed in hydrogel micro-structuring that involves the controlled freezing of a hydrogel solution to create intricate structures. During the process, ice crystals form within the hydrogel, guiding the spatial arrangement of the hydrogel components. Upon subsequent freeze drying, the ice crystals are removed, leaving behind a porous hydrogel scaffold with well-defined micro-structures [158].

Each method, which is adapted to particular applications, makes a contribution to the development of hydrogel micro-structuring, which in turn opens up several opportunities for the development of unique biomedical and biotechnological solutions. However, each method

mentioned above has advantages and limitations compared to each other, and all of these are summarized in Table 1.

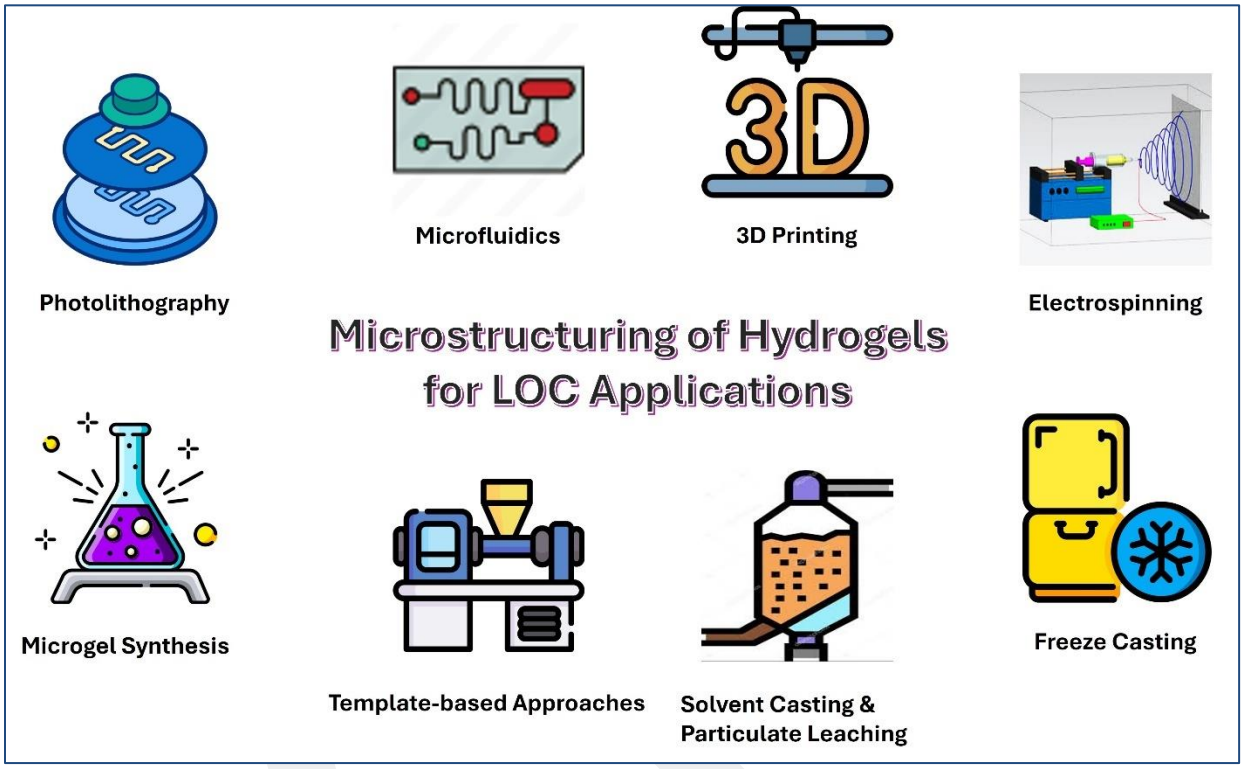


Figure 3. Advanced techniques for hydrogel micro-structuring for LOC applications.

Table 1. Advantages and limitations of hydrogel micro-structuring and integration into microfluidic system.

Micro-structuring Methods	Advantages	Limitations
Photolithography	• facilitate the extremely parallel construction of inter-connected structures [159] • is precise, time-efficient, inexpensive, and manageable in mass production [160] • allow construction of micro-nanostructures with extreme precision at the nanoscale [161]	• is challenging to fabricate 3D patterned structures [162] • is not compatible with the conventional techniques since the structures obtained by other methods cannot adjust to the dimensions (below 100 nm) of those produced by photolithography [163] • has an operation process consisting of long and complex recipes, and the initial installation equipment and routine maintenance of these equipment are expensive [164]
Microfluidics	• provide precise control, versatility, and compatibility with other fabrication methods [165] • allow for the production of monodisperse hydrogel microstructures, facilitating the creation of identical micro-tissues [166]	• have one major challenge occurrence of clogging and the limited architectural features, particularly in creating specific structures such as spheres, core-shell configurations, and fibers [167] • need for expensive specialized equipment that limits the fabrication of hydrogel microstructures [115]

3D Printing	• is more convenient, faster and more cost-effective than other techniques, and design freedom provides more innovative products [168] • enables the application of complex geometries and the production of desired objects in a short time [169] • reduces human intervention [170]	• suffers from the limited choice of materials available for the production of functional devices [171] • limits the finding or developing hydrogel formulations that can be printed while maintaining the desired properties [172] • may have limitations in achieving high-resolution structures in hydrogels due to factors such as print resolution, nozzle size, layer thickness, and rheological properties of the hydrogel ink [173] • may often require post processing such as cross-linking to achieve the desired mechanical properties and stability after printing [174]
Electrospinning	• ability to produce nanoscale to microscale fibers, mimicking the natural extracellular matrix and offering a high surface area [175] • facilitates the generation of porous and interconnected networks within hydrogels, enhancing their mechanical properties and promoting cell infiltration [176] • allows for the fabrication of multi-dimensional scaffolds consisting of nano and micropatterns, offering convenience, cost-effectiveness, and an easy procedure [177]	• may damage to the hydrogel's bioactivity and mechanical properties during the electrospinning process due to high voltage and the resulting fibers has poor mechanical strength [178] • makes the process sensitive and challenging to control parameters, including solution viscosity and conductivity [179] • has a limited capacity for material processing and is unable to process all hydrogels; nevertheless, it is able to process hydrogels that are sensitive to electric field [180]

Microgel Synthesis	• is simple, easy to functionalize, and sizes can be tuned from tens of nanometers to several micrometers [181] • has high control over cross-linking density through covalent cross-links between polymer chains [182] • can be obtained with low polydispersity via emulsion polymerization without using detergents, making their synthesis and working up easier [183]	• possess limitations in inorganic content, challenges in controlling colloidal stability, complexity in reaction network [184] • have scalability issues, and non-uniform formation under certain synthesis conditions [185] • can result in additional fabrication process due to the need of sophisticated technology [186]
Template-based Approaches	• provide precise control over hydrogel microstructures, allowing the incorporation of defined architectures such as channels, pores, and gradients [187] • have standardized templates ensures reproducibility, facilitating consistent properties across different studies [188] • possess the ease of fabrication and potential integration with other technologies [189]	• may limit the geometric complexity of hydrogel structures based on the usage of specific molds or templates and often result in single-step processes, restricting the creation of layered or multi-component designs [190] • have difficulties in template removal without damage yielding non-homogeneity throughout the hydrogel [191] • may have material compatibility issues, potential waste, and time-consuming processes [192]

Solvent Casting and Particulate Leaching	• provides precise control over the porosity and architecture of hydrogels by using particulate materials to create interconnected pores [193] • is relatively simple and cost-effective processes, making them accessible for researchers and practitioners [194] • provides the choice of particulate materials, enabling the customization of mechanical and structural properties of the hydrogel [195]	• can be time-consuming, and there is a risk of incomplete removal of particulates, which may compromise the structural integrity of the hydrogel [195] • may pose environmental and/or biocompatibility issues due to the solvent selection [196] • present difficulties in scaling up and mass production [197]
Freeze-Casting	• enables the production of hydrogels with tailored porosity, pore size, and overall architecture, making it suitable for applications in tissue engineering and regenerative medicine [198] • offers a unique advantage by allowing the incorporation of various materials, such as nanoparticles or cells, into the hydrogel matrix, leading to enhanced functionality [199]	• has potential for structural heterogeneity within the hydrogel scaffold due to the non-uniform distribution of ice crystals during the freezing process [200] • may be time-consuming, and controlling the freezing conditions to obtain precise and reproducible structures requires careful optimization [201]

5. Applications of Smart Hydrogels in LOC Systems

Smart hydrogels have found diverse applications in LOC systems, ranging from drug delivery and tissue engineering to microfluidic flow control, energy-efficient smart windows, and high-throughput analysis of cellular functions [202]. Their versatility and responsiveness make them valuable components in the development of advanced and multifunctional LOC devices [59]. Here, we tried to explain these diverse application areas of hydrogels by grouping them under six main headings: sample pre-concentration and enrichment, biomolecule separation and analysis, cell culture and organ on a chip models, controlled drug release, point of care diagnostics, bio-sensing. For immunoassays, smart hydrogels can be engineered to target specific biomolecules such as proteins, antibodies, and antigens, facilitating highly sensitive and selective detection [203]. In organ-on-a-chip applications, smart hydrogels mimic the extracellular matrix, providing a suitable environment for the culture and study of various tissues, including liver, heart, lung, and kidney models. This allows for realistic in vitro simulations of organ functions and disease states [204]. In sample pre-concentration and enrichment, hydrogels are utilized to selectively capture and concentrate low-abundance analytes from complex biological samples, enhancing detection sensitivity [205]. Biosensing applications leverage the responsive nature of hydrogels to detect changes in environmental conditions or the presence of specific target molecules, making them ideal for real-time monitoring [206]. For drug delivery, hydrogels can be designed to release therapeutic agents in a controlled manner, responding to specific physiological triggers, thereby improving the efficacy and safety of treatments [207]. In personalized medicine, the customizable properties of hydrogels enable the development of tailored therapeutic strategies that meet the unique needs of individual patients [208]. The most current literature examples of these applications are presented in Table 2.

Table 2. Applications of smart hydrogels in LOC systems.

Materials of Hydrogel	Type of Stimuli	Purpose of Use	Chip Type	Application Area	Ref
PNIPAAM	thermo-responsive	to develop microfabricated smart hydrogel platforms to compressively mold microtissues such as spheroids or organoids into customized forms, on demand	3D printed chip	cell culture and organ on chip application	[209]
PNIPAAM+Fe_3O_4 NPs.+GO+Alg	light+magneto +pH multi-responsive	to develop a novel multiresponsive drug delivery system	centrifugal microfluidic chip	controlled drug release	[210]
Col I+ AgNWs	electro-responsive	to release nanomaterials with the electrical stimuli and to target brain tumor cell	microfluidic actuator platform	controlled nanomaterials release for photo-thermal therapy	[211]

MXene+PDA+GelMA	optical and electrical dual responsive	to create a responsive and biomimetic environment	PDMS based microfluidic platform	bio-sensing + cell culture and organ on chip applications	[212]
CS	pH responsive	to create a sensor layer filled with pH sensitive hydrogel	PDMS based microfluidic platform	bio-sensing	[213]
PEG+PNIPAAM+PCL	thermo-responsive	to enable the retrieval of uniform sized cell spheroids into hydrogel environment below the LCST	PDMS based microfluidic platform	cell culture and organ on chip applications	[214]
Fe^{3+}-Alg	electro-responsive	to achieve the release of Fe ions from hydrogels by an external electrical signal through an integrated gold plate electrode	PMMA based microfluidic platform	drug delivery	[215]
PNIPAAm+BIS+BAC	redox-responsive	to enable the dynamic capture and release of various different functionalized (macro)molecules (e.g., proteins and drugs)	PDMS-on-glass microfluidic device	bio-sensing + drug delivery	[41]
AcAM (Acrylamid)	photo-responsive	to enhance the functionality of PDMS microfluidic devices by optimizing the polymerization process of nanoporous hydrogels, hence enabling a faster and more straightforward method	PDMS-on-glass microfluidic device	analyte enrichment	[216]

PEGDA	UV-responsive	to generate tumor cell spheroids and to propose a system for preclinical drug resistance evaluation	PDMS based microfluidic chip	cell culture and organ on chip application	[217]
COL/Fb (Fibrin)	temperature-responsive	to report microfluidic micro-fluidic organ-on-chip model of outer blood retinal barrier that pigment epithelium and a micro-vessel of human endothelial cells, separated by a semi permeable membrane	PDMS based microfluidic chip	cell culture and organ on chip application	[218]
GO/His	electrical-responsive	to create a biocompatible, electrically conductive, adaptable, and functional material that can be used in a variety of industries, including electronics and biomedicine.	3D printed microfluidic device	cell culture and organ on chip application	[219]
PNIPAAM+ Fe_3O_4 Np.	electrical-responsive + thermo-responsive	to prepare a hydrogel that carries and releases a controlled and targeted drug	Droplet-based microfluidic chip	controlled drug delivery	[220]
Col/PEGDA/Alg	thermo-responsive	to create a supportive and biomimetic environment for cell growth and organization within the microfluidic system	3D printed T-junction based microfluidic chip	cell culture and organ on chip application	[221]

GelMA-BP-Mg	pH-responsive	to develop a hydrogel system that captures and releases magnesium ions, a trace element that has a significant impact on human metabolism and tissue regeneration.	droplet based microfluidic chip	cell culture and organ on chip application	[222]
Gelatin	UV-responsive	to enhance the retention of extracellular vesicles and sustainably release them at therapeutic targets	droplet based microfluidic chip	point of care diagnostic and biosensing	[223]
CS-Mo (Chitosan-Molibdenum)	electro-responsive	to develop a portable, cost-effective sensor-containing device that can be used for rapid detection and monitoring of post-harvest volatile biomarkers in food storage and ripening	micro-fabricated electrochemical chip	sample pre-concentration and enrichment + biosensing	[224]
PEGDA/GelMA	UV-responsive	to create modular cell embedded microstructures with intricate fine patterns, high cell viability, and assemble them for in vitro biomimetic tissue reconstruction	PDMS based optofluidic thin-film chip	cell culture and organ-on-chip applications	[225]
PAAm (polyacrylamide)	UV-responsive	to achieve rapid and sensitive hydrogel based high throughput cell screening with a low cost and simple strategy	microfluidic spotting-screening platform	cell culture and organ-on-chip applications	[226]

PEGDA	UV-responsive	to develop a platinum nanozyme/hydrogel composite based sensing system for one step stand-alone glucose detection	PEGDA based microfluidic chip	sample pre-concentration and enrichment + biosensing	[227]
calcium alginate	pH-responsive	to fabricate a small intestine-targeted drug delivery by oral administration	PDMS based microfluidic chip	cell culture and organ-on-chip applications	[228]
PNIPAAm+GO	thermo-responsive	to present a contactless and spatiotemporally controllable photo-thermal microfluidic pumping strategy for multi-layer biomolecule transportation	paper based microfluidic device	sample pre-concentration and enrichment	[229]
GelMA and thiolated hyaluronic acid (HA-SH)	UV-responsive	to develop a tissue-like microgel array to assess stem cell differentiation in complex and heterogeneous cell microenvironments	3D micro-print array	cell culture and organ-on-chip applications	[230]
NdFeB-hybrid GelMA-HAMA	Magneto-responsive	to investigate the inflammation-related genes and proteins in chondrocytes changed with mechanical stress stimulation in the cartilage-on-a-chip through magneto-responsive hydrogel	PDMS based microfluidic chip	cell culture and organ-on-chip applications	[231]

When the given literature examples were evaluated comparatively in Table 2, it becomes clear that different types of smart hydrogels offer distinct advantages and challenges, influencing their prevalence in LOC applications. pH-responsive and temperature-responsive hydrogels are among the most frequently used due to their relatively straightforward synthesis and well-understood mechanisms. pH-responsive hydrogels are particularly advantageous in biomedical applications where pH variations are a critical factor, offering precise control over drug release. However, their performance can be limited by the narrow range of effective pH and slower response times [232]. Temperature-responsive hydrogels, with their ability to undergo phase transitions at specific temperatures, are favored in cell culture and controlled drug release systems. They offer the advantage of reversible and controllable transitions, but require precise temperature regulation, which can be challenging in dynamic environments [233]. On the other hand, light-responsive and electro-responsive hydrogels provide high spatial and temporal control, essential for applications requiring non-invasive and remote manipulation. Light-responsive hydrogels are particularly useful in targeted drug delivery and actuation, though they require specific light wavelengths and careful consideration of phototoxicity. Electro-responsive hydrogels are advantageous in microfluidics and soft robotics due to their rapid response to electrical stimuli, yet their efficiency can be compromised by the ionic strength of the surrounding medium [234]. Multi-responsive hydrogels, although complex to synthesize, offer versatile applications by responding to multiple stimuli, providing enhanced control and adaptability. Their multifaceted responsiveness makes them highly suitable for advanced multifunctional LoC systems, despite the increased complexity in their design and characterization [235]. In conclusion, the choice of hydrogel type in LoC systems is heavily dependent on the specific application requirements and the inherent properties of the hydrogels [236]. pH- and temperature-responsive hydrogels are widely used due to their simplicity and reliability, whereas light- and electro-responsive hydrogels offer precise control but require careful handling of external stimuli [237]. Multi-responsive hydrogels, though challenging to design, hold significant promise for future multifunctional applications [238–240].

6. Characterization and Evaluation of Smart Hydrogel-based LOC Devices

The comprehensive characterization and evaluation of smart hydrogels encompass a meticulous assessment of various physical, chemical, and responsive properties that are crucial in understanding their behavior and optimizing their performance [241]. To accomplish this, a multitude of methods and parameters have been developed and employed, each offering unique

insights into the intricate nature of these intelligent materials [242]. Within this scope, characterizations are classified into physical, chemical, and biological categories. While physical characterizations include swelling ratio, mechanical properties, stimuli-responsiveness, thermal properties, and electrical conductivity, chemical characterizations encompass crosslinking density, chemical composition, release kinetics, and degradation and stability. Meanwhile, biological characterizations cover biocompatibility and morphology. Details on these characterizations are provided below under their respective categories.

Swelling Ratio: The swelling ratio is a crucial parameter employed in the characterization of smart hydrogels. It plays a significant role in determining the extent of hydration or expansion demonstrated by the hydrogel when subjected to particular stimuli [243]. This parameter serves as a reliable indicator, providing valuable insights into the behavior of smart hydrogels. The quantification of a crucial parameter is commonly achieved by calculating the ratio between the volume of a hydrogel in its swollen state and its initial dry volume. This approach allows for a quantitative assessment of the hydrogel's responsiveness and its capacity to undergo volumetric changes [244].

Mechanical Properties: The evaluation of mechanical properties plays a crucial role in assessing the potential applications of smart hydrogels. Specifically, the elasticity and stiffness characteristics of these materials are of significant importance. These properties directly influence the performance and functionality of smart hydrogels in diverse fields. In order to comprehensively understand the characteristics of hydrogels, it is imperative to employ sophisticated techniques such as rheology or compression testing. These advanced methods facilitate a thorough assessment of the hydrogel's response to external forces and its ability to endure mechanical stress. By utilizing these techniques, researchers can gain valuable insights into the mechanical properties of hydrogels, enabling a more comprehensive understanding of their behavior under various conditions [245].

Stimuli Responsiveness: The investigation of hydrogels' stimuli-responsiveness has been a subject of great interest in recent years, as it directly influences their functionality and potential applications. Among the various environmental factors that can affect hydrogels, pH, temperature, and ions have emerged as key determinants of their responsiveness [246]. The pH sensitivity of hydrogels has been extensively studied due to its relevance in biomedical and drug delivery applications. Hydrogels can exhibit pH-dependent swelling behavior, where changes in the

surrounding pH alter their volume and properties. This responsiveness is attributed to the presence of ionizable groups within the hydrogel network, which can undergo protonation or deprotonation in response to pH variations [81]. By carefully designing the composition and structure of hydrogels, researchers have been able to achieve precise control over their pH responsiveness, enabling applications such as pH-triggered drug release or pH sensors [232]. Temperature responsiveness is another crucial aspect of hydrogel functionality in a hydrogel system requires the quantification of alterations in volume, mass, or other physical characteristics in reaction to deliberate temperature modifications in the surrounding environment. Assessments of the hydrogel's interactions with its surrounding environment are crucial for obtaining a comprehensive understanding of its behavior and unlocking its potential applications. These evaluations offer valuable insights that contribute to the advancement of hydrogel technology [247].

Thermal Properties: The thermal properties of smart hydrogels play a crucial role in numerous applications, making them a significant area of focus. Among these properties, the temperature at which phase transitions occur stands out as a particularly important factor. Understanding and manipulating these thermal characteristics are essential for optimizing the performance and functionality of smart hydrogels in various fields [248]. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are widely recognized as indispensable techniques for characterizing the thermal properties of hydrogels [249]. These techniques provide valuable insights into the behavior of hydrogels across a range of temperature conditions, facilitating the optimization of their performance. By employing DSC and TGA, researchers can gain a comprehensive understanding of the thermal stability, phase transitions, and decomposition behavior of hydrogels [250]. Consequently, DSC and TGA have emerged as essential tools in the field of hydrogel research, enabling scientists to make informed decisions regarding the design and development of advanced hydrogel materials.

Electrical Conductivity: The assessment of electrical conductivity is of utmost importance when considering the design of an electrically conductive hydrogel [251]. In the field of hydrogel research, various techniques have been developed to assess the electrical conductivity such as impedance spectroscopy and four-probe conductivity measurements [252]. Consequently, by employing these non-destructive techniques, researchers are able to obtain valuable data that sheds light on the conductivity of hydrogels, thereby enabling a comprehensive evaluation of their suitability for use in electronic or sensing devices [253].

Crosslinking Density: The mechanical and responsive properties of hydrogel networks are greatly influenced by the crosslinking density, making it a crucial parameter to consider. In the field of hydrogel research, the evaluation of crosslinking density is of paramount importance as it provides valuable insights into the internal structure and subsequent behavior of these materials [254]. To this end, various techniques have been utilized, with small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) spectroscopy being the most commonly employed methods [255]. SAXS, a powerful characterization tool, enables the determination of the spatial arrangement of polymer chains within the hydrogel network. By analyzing the scattering patterns of X-rays passing through the sample, valuable information regarding the average distance between crosslinks and the distribution of chain conformations can be obtained. This technique offers a non-destructive and non-invasive means of assessing the crosslinking density, allowing researchers to gain a deeper understanding of the hydrogel's internal structure [256]. Similarly, NMR spectroscopy has proven to be an invaluable tool in the evaluation of hydrogel crosslinking density. By exploiting the unique magnetic properties of atomic nuclei, NMR spectroscopy provides detailed information about the molecular structure and dynamics of hydrogel networks. By analyzing the NMR spectra, researchers can deduce the extent of crosslinking and gain insights into the mobility and interactions of polymer chains within the hydrogel matrix [257]. Both SAXS and NMR spectroscopy offer distinct advantages in the assessment of hydrogel crosslinking density. SAXS provides valuable information about the overall arrangement of polymer chains, while NMR spectroscopy offers insights into the molecular-level interactions and dynamics [258]. By employing these techniques, researchers can gain a comprehensive understanding of the hydrogel's internal structure, paving the way for the development of improved materials with tailored properties and enhanced performance.

Chemical Composition: In order to enhance our understanding of the chemical composition of hydrogels, various techniques have been reported up to date. Fourier Transform Infrared Spectroscopy (FTIR) identifies functional groups, Nuclear Magnetic Resonance (NMR) provides insights into molecular structure, and UV-Visible Spectroscopy analyzes optical properties [259]. X-ray Photoelectron Spectroscopy (XPS) assesses surface composition, and Gel Permeation Chromatography (GPC) determines molecular weight distribution while Raman Spectroscopy examines molecular vibrations [259]. Mass Spectrometry, including MALDI-MS and ESI-MS, analyzes molecular weight and structure of the hydrogels [260]. Lastly, Zeta Potential Measurement determines the surface charge of the hydrogels [261]. Integrating these techniques

offers a comprehensive understanding of hydrogel composition, aiding in the optimization of formulations for specific applications in biomedicine and materials science.

Release Kinetics: In the field of hydrogel-based drug delivery systems, the investigation of release kinetics is of utmost importance [262]. Measuring the release kinetics of hydrogels in drug delivery is especially crucial for assessing the controlled release profile of the therapeutic agents. One commonly employed method is to conduct *in vitro* drug release studies under physiological conditions [263]. For this purpose, hydrogels are immersed in a release medium, often simulating body fluids, and the concentration of the released drug is periodically analyzed using techniques such as high-performance liquid chromatography (HPLC), UV-Visible spectroscopy, or mass spectrometry [264]. These analyses provide quantitative data on the temporal release of the drug from the hydrogel matrix. Mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, can be applied to interpret the release kinetics and understand the underlying mechanisms [265]. These models help elucidate whether drug release follows a specific pattern, such as diffusion-controlled release, erosion-controlled release, or a combination of both [266]. Additionally, factors like hydrogel composition, crosslinking density, and drug loading capacity influence release kinetics and must be carefully considered during the design and characterization of hydrogels for drug delivery applications [267]. Overall, a combination of experimental studies and mathematical modeling provides a rigorous approach to understanding and optimizing the release kinetics of hydrogels for effective and controlled drug delivery [268].

Degradation and Stability: It is crucial to thoroughly investigate the degradation and stability of hydrogels over time as part of their comprehensive characterization [269]. In order to achieve a comprehensive understanding of the long-term behavior of hydrogels and optimize their stability and durability, researchers have conducted numerous studies involving exposure to relevant environmental conditions and monitoring changes in mass and structure over time [270]. In other words, by closely monitoring changes in mass and structure over extended periods, researchers have been able to discern patterns and trends that provide insights into the degradation mechanisms and potential failure modes of hydrogels [271]. Overall, the utilization of exposure studies and long-term monitoring techniques has significantly contributed to the advancement of hydrogel research and has paved the way for the design and fabrication of more robust and reliable hydrogel materials [272].

Biocompatibility: In the realm of hydrogels designed for biological applications, the evaluation of their biocompatibility holds significant importance. In order to assess the biocompatibility of hydrogels with living systems, a range of biocompatibility assays have been developed and employed [273]. Cell viability and proliferation assays, such as MTT and Live/Dead staining, determine the biocompatibility of hydrogels by examining cell response and growth [274]. Evaluating cell adhesion and morphology through techniques like immunofluorescence and scanning electron microscopy (SEM) provides insights into the material's ability to support cellular attachment and spreading [275]. Cytotoxicity assays, including lactate dehydrogenase release, gauge potential toxic effects on cells, while studies on inflammatory responses assess the hydrogel's immunological impact [275]. Hemocompatibility testing of hydrogels evaluates interactions with blood components for applications involving blood contact [276]. For tissue engineering, assessments include cell differentiation studies, angiogenesis potential, and in vivo studies to determine biocompatibility and tissue integration [277]. Hydrogels designed for drug delivery are characterized by drug release kinetics and the bioactivity of released substances [278]. This collective approach to biological characterization informs the development of hydrogels for diverse biomedical applications, ensuring their suitability for specific therapeutic contexts.

Morphology: Various characterization techniques are employed to analyze the morphology of hydrogels. Optical microscopy, including bright-field and fluorescence microscopy, provides a simple means of visualizing the overall structure of hydrogels [279]. SEM and transmission electron microscopy (TEM) offer high-resolution imaging of the surface and internal morphology of hydrogels at the nanoscale [258]. Atomic force microscopy (AFM) allows for nanoscale surface topography studies, which is particularly suited for soft materials like hydrogels [280]. X-ray diffraction (XRD) can assess the crystallinity and structural arrangement of hydrogels [281]. Nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR) provide insights into the molecular structure and composition of hydrogels [282]. Confocal laser scanning microscopy (CLSM) can generate three-dimensional images of hydrogels [283]. Rheological analysis is used to examine the mechanical properties of hydrogels [284]. Small-angle X-ray scattering (SAXS) studies the nanostructure of hydrogels, providing information on size, shape, and arrangement of structural elements [285]. By combining these techniques, a comprehensive understanding of hydrogel morphology can be obtained, which is crucial for tailoring their properties for specific applications.

In this review, we have highlighted the significance of employing diverse methods in the characterization of smart hydrogels. The selection of characterization methods is contingent upon the unique characteristics and desired uses of the smart hydrogel. In order to obtain a comprehensive understanding of a hydrogel's behavior under various conditions, it is often necessary to employ a combination of different techniques. By utilizing multiple techniques, researchers can gather a wide range of data and observations, enabling them to draw more accurate conclusions about the hydrogel's behavior, paving the way for advancements in fields such as biomedicine, drug delivery, tissue engineering, and environmental science.

Current Challenges and Future Directions

Current challenges in the use of smart hydrogels in LOC applications can be summarized under the headings of optimizing response times, stability durability, compatibility with diverse analytes, synthesis fabrication techniques and interdisciplinary collaboration [286,287]. Achieving rapid response times remains a challenge for many smart hydrogel systems, especially in real-time applications for LOC devices [288]. Ensuring stability and long-term durability of smart hydrogels is also a persistent challenge, with factors such as mechanical stability, resistance to degradation, and long-term functionality needing to be addressed for practical and reliable applications [289]. Smart hydrogels often face challenges in being universally compatible with a wide range of analytes, and developing hydrogels that exhibit responsiveness across various chemical and biological substances is essential for versatile LOC applications [290]. Refining synthesis methods and fabrication techniques for smart hydrogels is an ongoing challenge, with the need to achieve reproducibility, scalability, and cost-effectiveness in production materials for widespread adoption [291]. Bridging the gap between materials science, engineering, and biology through interdisciplinary collaboration is essential to overcome these challenges and optimize the performance of smart hydrogels in LOC applications [292].

The development of smart hydrogel-based applications in LOC applications can be advanced in several key areas such as advanced sensing capabilities, real-time monitoring, enhanced biocompatibility for medical applications, integration with emerging technologies, and responsive nanomaterials [293]. Firstly, there is a need to enhance the sensing capabilities of smart hydrogels to enable more precise and diverse analyte detection [294]. This can be achieved by incorporating advanced sensor technologies and exploring new responsive mechanisms, which can contribute

to heightened sensitivity and specificity in analyte detection [53]. Additionally, the focus should be on developing smart hydrogels capable of real-time monitoring in microfluidic systems, as this holds promise for achieving instantaneous responses to environmental changes, thereby significantly improving the dynamic control efficiency of the LOC device [295]. Furthermore, future smart hydrogels should prioritize achieving higher biocompatibility to expand their applications in medical diagnostics, drug delivery, and personalized medicine. Designing hydrogels that are compatible with biological systems is essential for advancing healthcare-related LOC technologies [296]. Moreover, the integration of emerging technologies such as artificial intelligence (AI) and machine learning can enhance the intelligent decision-making capabilities of smart hydrogel-based LOC devices, potentially leading to the development of autonomous adaptive systems [297]. Lastly, the exploration of incorporating responsive nanomaterials into smart hydrogels offers additional functionalities to tailor their response properties, thereby opening new possibilities for improving the overall performance of smart hydrogel-based systems [296]. These advancements are crucial for the development of more efficient and versatile LOC devices with applications in various fields, including healthcare and environmental monitoring.

Conclusion

Smart hydrogels play pivotal roles in LOC technology by enabling responsive sensing and actuation functionalities. These hydrogels contribute to real-time monitoring, offering heightened sensitivity and adaptability to environmental changes within microfluidic systems. Their advanced sensing capabilities, coupled with the potential for integration with emerging technologies like AI, enhance decision-making within LOC devices. Moreover, the versatility of smart hydrogels extends to medical applications, where their biocompatibility facilitates advancements in diagnostics and drug delivery. The incorporation of responsive nanomaterials further tailors their properties, opening new possibilities for nanoscale applications. Overall, the key contributions of smart hydrogels lie in their dynamic responsiveness, real-time monitoring capabilities, and diverse applications within the burgeoning field of LOC technology. Widespread adoption of smart hydrogel-based LOC devices is promising due to several compelling factors. The dynamic responsiveness of smart hydrogels to environmental stimuli enhances the adaptability and functionality of LOC systems, making them versatile across various applications. The potential for real-time monitoring and precise control addresses critical needs in fields such as healthcare, diagnostics, and environmental monitoring. Additionally, the integration of smart hydrogels with

emerging technologies, including artificial intelligence and machine learning, offers intelligent decision-making capabilities, further advancing the utility of LOC devices. The biocompatibility of smart hydrogels expands their use in medical applications, opening doors to personalized medicine and drug delivery. Challenges in optimization and fabrication techniques are being actively addressed, paving the way for improved stability and reproducibility. As interdisciplinary collaboration continues to flourish, fostering partnerships between materials science, engineering, and biology, the prospect for the widespread adoption of smart hydrogel-based LOC devices becomes increasingly feasible, heralding a new era in sensing, diagnostics, and beyond.

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Conflict of Interest

Authors declare that there is no conflict of interest.

Author Contributions

Atakan Tevlek designed the content of the article. Atakan Tevlek and Esin Akbay Çetin performed literature survey and wrote the article. The acknowledged people helped in literature survey. Berfin Sağlam, Doğa Yazar and Atakan Tevlek prepared the figures.

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