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Biomaterials in droplet-based microfluidics: From structural design to biomedical applications

Andrea Fergola [a,1], Cesare Gabriele Gaglio [a,1], Simone Luigi Marasso [a,b], Matteo Cocuzza [a,b], Candido Fabrizio Pirri [a], Lucia Napione [a], Francesca Frascella [a,*]

[a] Department of Applied Science and Technology (DISAT), Politecnico di Torino, 10129, Torino, Italy
[b] Consiglio Nazionale delle Ricerche, Istituto dei Materiali per l'Elettronica ed il Magnetismo (CNR IMEM), 43124, Parma, Italy

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ABSTRACT

Droplet-based microfluidics (DBM) affords reproducible control over the breakup of immiscible streams, enabling the on-demand fabrication of well-defined carriers for biomedical use. We first outline droplet-generation techniques, distinguishing passive architectures—in which capillary, viscous, and inertial forces set size and frequency—from active methods that superimpose external fields to refine monodispersity, throughput, and size control. Building on this physical framework, we survey the micro- and nanostructures accessible with DBM—including polymeric nanoparticles and nanogels/microgels, microspheres, core–shell microcapsules, and microfibers—and show how morphology (porosity, shell thickness, network architecture) and spatial composition govern transport, stability, and release. We then examine the biomaterials that endow droplets with function, with emphasis on natural, semi-synthetic, and synthetic hydrogels and on gelation/polymerization routes (ionic, thermal, photo-induced, enzymatic) that are compatible with biological cargo and permit real-time structural control. The applications analysis is intentionally biomaterial-centric. For drug delivery, we relate material choice and crosslinking chemistry to representative release profiles and kinetic models, and we integrate quantitative biocompatibility readouts where available (e.g., LD_{50}, inflammatory signaling) together with in vivo biodistribution and loading efficiency that link carrier design to payload fate. For cell-centric uses, we discuss single-cell encapsulation and droplet-based 3D cultures, highlighting biomaterial-driven morphogenesis, viability, and function, and we outline DBM-enabled bioanalytical platforms (single-molecule detection, single-cell sequencing). By articulating the pathway from droplet-generation techniques, through the resulting micro-/nanostructures and the selected biomaterials, to their biomedical performance, this review provides a coherent design perspective for engineering DBM-fabricated carriers and scaffolds in drug delivery, tissue modeling, and high-throughput bioanalysis.

1. Introduction

Droplet-based microfluidic (DBM) systems enable the generation, manipulation, and processing of discrete fluid volumes within immiscible phases, such as oil-in-water (O/W) or water-in-oil (W/O) droplets [1–3]. By exploiting the interplay between hydrodynamic forces and interfacial tension, continuous fluid streams are segmented into discrete droplets dispersed within a carrier fluid [4,5]. This allows precise control over droplet size, composition, and production frequency, supporting high-throughput experiments with enhanced mixing and reaction efficiency [6]. Traditional droplet generation techniques—bulk emulsification [7], air jetting [8], electro-hydrodynamic generation [9,10], and centrifugal microfluidics [11]—have inherent limitations [12]. Bulk emulsification allows large-scale droplet production but suffers from broad size distributions and poor reproducibility. Air jetting can achieve high throughput but often deforms droplets and destabilizes interfaces. Electro-hydrodynamic approaches enable fine size control through electric fields but require careful tuning and may stress sensitive biological cargo. Centrifugal or spinning-disc methods allow parallelization but offer limited precision in droplet composition and generation frequency. DBM overcomes these limitations by producing highly controlled and reproducible droplets. The injection of immiscible fluids

* Corresponding author.
E-mail address: francesca.frascella@polito.it (F. Frascella).
[1] These authors contributed equally as co-first authors.

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into microchannels forms continuous and dispersed phases, with droplet formation driven by the balance between viscous drag and interfacial tension, or by pressure-driven breakup when the dispersed fluid plugs the channel [6,13]. The combination of this precise control, high surface-area-to-volume ratios and rapid mixing has enabled a variety of applications, including drug delivery [14], emulsification [15], synthesis of nano- and microparticles [16], and the encapsulation of biological material [17,18], thus supporting controlled biochemical or chemical reactions [3]. Fig. 1 displays a comparative bibliometric analysis of the number of publications indexed in PubMed from 2000 to 2025 concerning the main droplet generation techniques. While traditional approaches have remained relatively stable with limited growth, droplet-based microfluidics exhibits a marked exponential increase, confirming its establishment as the reference technology in the field. The figure also includes a comparative summary table outlining the main advantages and limitations of each technique, underlining how DBM uniquely combines high precision, reproducibility, and biocompatibility with broad applicability.

foaming [30], salt leaching [31], and emulsion-based microparticles [32]—have demonstrated the ability to generate porous 3D constructs. However, they often result in inhomogeneous architectures, limited pore interconnectivity, or require harsh solvents. In contrast, DBM allows the generation of highly uniform microdroplets that can be assembled into constructs with precisely controlled architecture, pore size, and mechanical properties [1,2]. The integration of biomaterial-laden droplets into tissue engineering applications unlocks a broad spectrum of biomedical functionalities. DBM supports cell encapsulation and culture within microscale niches, enabling proliferation, differentiation, and the formation of organized tissue. Beyond tissue engineering, DBM underpins lab-on-chip and organ-on-chip systems, where droplets replicate physiological microenvironments for high-throughput screening, phenotypic assays, and toxicological studies [13,33,34]. Sequential droplet handling further extends to genomic analyses, including digital PCR [35], single-cell RNA sequencing, and ChIP-seq, enabling reproducible and multiplexed workflows at the single-cell level [36,37]. In addition, DBM provides modular building

Technique	*DBM*	*Bulk Emulsification*	*Air Jetting*	*EHD*	*Centrifugal*
Strengths	High precision, reproducibility, versatility, biocompatibility	Easy, very scalable, low cost	Simple, scalable, versatile	Precise, reproducible, versatile	Scalable, simple, reproducible
Weaknesses	Complex setup, limited scalability	Poor precision, low reproducibility, low biocompatibility	Low precision, moderate reproducibility	Complex, biocompatibility issues, low scalability	Lower precision, limited versatility

Within DBM systems, droplets act as uniform microreactors that provide confined and well-defined microenvironments for hosting biomaterials [19]. By tailoring their structural and chemical properties, droplets can sustain cell viability, guide differentiation, and promote tissue formation, thereby linking droplet fabrication with tissue engineering and regenerative medicine [20,21]. In this context, both natural and synthetic polymers have been extensively investigated as scaffolding materials. Natural polymers—including collagen [22], alginate [23], and hyaluronic acid [24]—offer intrinsic biocompatibility, biodegradability, and similarity to the extracellular matrix (ECM) [25]. Synthetic polymers, on the other hand, enable precise control of mechanical properties and degradation rates, making it possible to fine-tune scaffold behavior [26]. Conventional scaffold fabrication methods—such as electrospinning [27,28], freeze-drying [29], gas

blocks for the fabrication of functional nano- and microstructures, which can be assembled into 3D constructs for regenerative medicine [38]. Overall, DBM-generated biomaterials offer a versatile and precise platform to engineer advanced tissue constructs, design-controlled drug delivery systems, and support next-generation lab-on-chip applications. Despite this potential, technical challenges in droplet generation and biomaterial handling still represent significant barriers to full clinical translation. Recent advances in droplet-based microfluidics techniques have addressed several longstanding limitations, such as the production of monodisperse droplets containing stable biomaterial formulations, enhancing assay reproducibility, and significantly improving the scalability and efficiency of production workflows [3,21]. Despite these technical improvements, a recent analysis of the biomaterials used, their structural design, and the advantages they confer is still lacking. Most

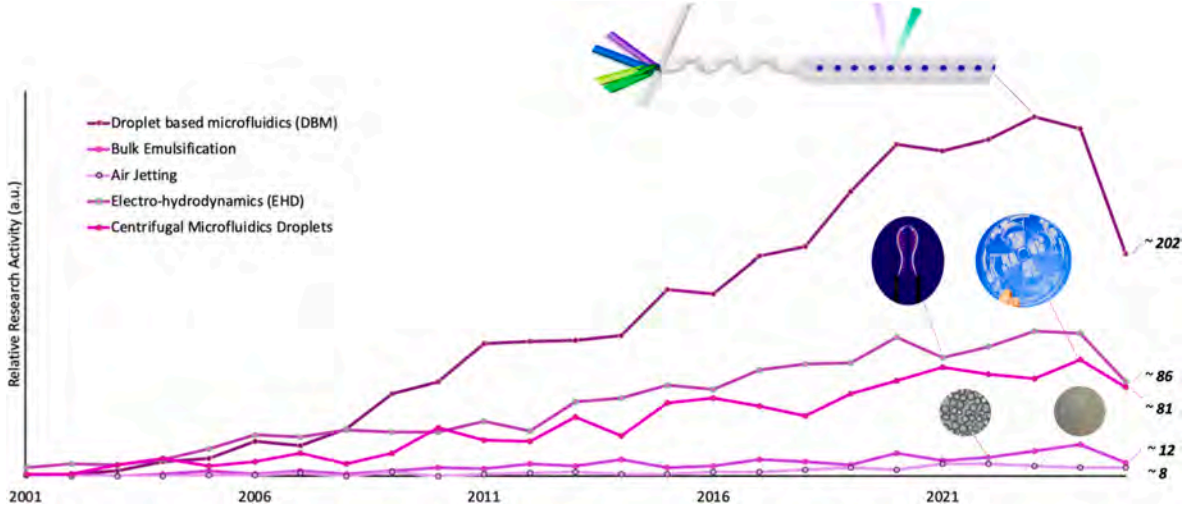

Fig. 1. Publication trends in droplet generation techniques (2000–2025). PubMed-indexed articles on bulk emulsification, air jetting, electro-hydrodynamic (EHD), centrifugal, and droplet-based microfluidics (DBM). DBM shows a sharp rise compared to the relatively stable output of traditional methods. A summary of the main advantages and limitations of each technique is also provided.

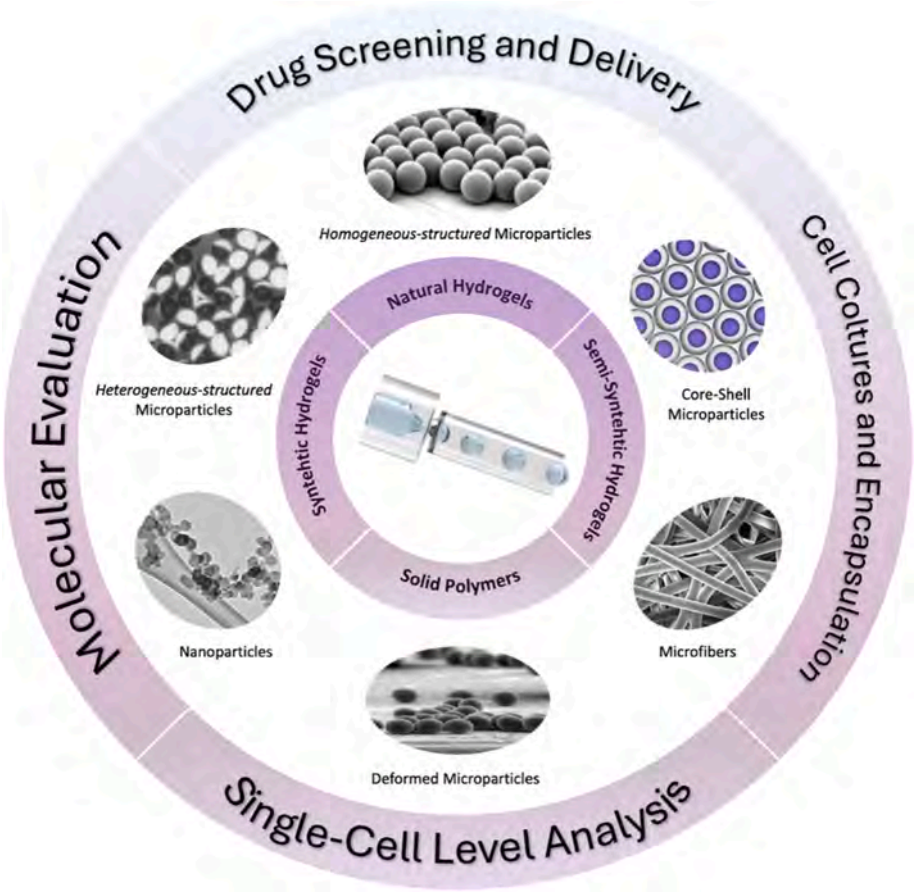

Fig. 2. Starting from droplet-based microfluidic devices at the center, the image outlines the types of biomaterials used (solid polymers and hydrogels), the range of resulting particle architectures, and their main biomedical applications.

reports focus on droplet generation or simple encapsulation, without fully exploring how the choice of biomaterial can expand the range of achievable microarchitectures, functionalities, and applications. This gap limits the potential of DBM to produce more complex and versatile biomedical systems, such as tailored scaffolds, advanced drug delivery carriers, and high-throughput cell culture platforms. In this review (see Fig. 2), we focus specifically on biomaterials in DBM, aiming to provide researchers with guidance for selecting the most suitable material for a given biomedical application. We also analyze micro- and nano-structured systems, including polymeric nanoparticles, microspheres, microcapsules, and microfibers, highlighting both their morphological characteristics and the design strategies that underlie their formation. Several reviews outline DBM applications in the biomedical field [38,39]; however, to the best of our knowledge, an up-to-date focus on the biomaterials themselves could assist researchers in optimizing material selection for specific functional outcomes. For this reason, particular attention is devoted to natural hydrogels (e.g., alginate, gelatin, collagen, chitosan, agarose) and synthetic counterparts (e.g., Poly(ethylene glycol) (PEGDA), polyvinyl alcohol (PVA), polyacrylamide (PAAm)), along with the gelation mechanisms—chemical, physical, or enzymatic—used to tailor their mechanical and functional properties. Finally, we discuss the biomedical applications of DBM-fabricated biomaterials, ranging from drug delivery carriers and 3D scaffolds to microincubators for single-cell culture and high-throughput biological assays, highlighting how careful material selection and structural design can expand the versatility and translational potential of these platforms.

1.1. Droplet-based microfluidics principles

One of the most well-known and adaptable methods for creating micro- and nanoparticles is provided by the droplet-based microfluidic approach. The working principle of these devices is based on interfacial phenomena that are common at the microscale, where bulk forces are subordinated to surface forces. When two partially or completely miscible fluids come into contact at a microfluidic interface, controlled droplet formation takes place. At this point, interfacial tension acts to minimize the contact area between the fluids, while viscous stresses deform the interface and drag it along the channel, leading to controlled break-up of the dispersed phase and formation of uniform droplets [40]. Key characteristics including droplet size, generation frequency, and internal composition may be precisely controlled with microfluidic droplet generation devices. This high degree of tunability makes them ideal for advanced biomedical applications, facilitating the encapsulation of drugs, bioactive molecules or cells within highly reproducible microscopic carriers. The instability of the interface between a continuous and a dispersed flow provides the basis for droplet formation, which causes the inner phase to break into distinct droplets. Although the dynamics of this process can be modulated in a variety of ways, the behaviour of the fluids involved is generally described by dimensionless numbers that take into account the physical properties, flow conditions and geometry of the device [3,41].

1.1.1. Dimensionless parameter in droplet-based microfluidics

In a microfluidic system, multiple physical phenomena occur simultaneously, and their relative importance is expressed through dimensionless numbers, which are fundamental for characterizing the droplet formation mechanism (Table 1) [42,43].

Table 1
Dimensionless numbers relevant to droplet-based microfluidics and their physical interpretation.

Symbol	Name	Formula	Physical Meaning
Re	Reynolds number	$Re = \frac{\rho u L}{\eta}$	Inertial force/viscous force
Ca	Capillary number	$Ca = \frac{\eta u}{\gamma}$	Viscous force/interfacial tension
We	Weber number	$We = \frac{\rho u^2 L}{\gamma}$	Inertial force/interfacial tension
Bo	Bond number	$Bo = \frac{\Delta \rho g L^2}{\gamma}$	Buoyancy/interfacial tension
λ	Viscosity ratio	$\lambda = \frac{\eta_d}{\eta_c}$	Dispersed viscosity/Continuous viscosity
φ	Flow rate ratio	$\varphi = \frac{Q_d}{Q_c}$	Dispersed flow rate/Continuous flow rate

The Reynolds number (*Re*) expresses the ratio between inertial and viscous forces:

$$Re = \frac{\rho u L}{\eta} \tag{1}$$

where ρ is the fluid density, u is the flow velocity [m/s], L is a characteristic length of the device, and η is the dynamic viscosity. In microfluidic systems, *Re* is usually very low (10^{-6} – 10), indicating a laminar regime where viscous forces dominate.

To describe droplet formation, it is more common to refer to the Capillary number (*Ca*), which represents the ratio between viscous stress and capillary pressure:

$$Ca = \frac{\eta u}{\gamma} \tag{2}$$

where γ is the surface tension. In microfluidics, *Ca* typically ranges from 10^{-3} to 10.

Different *Ca* values give rise to three distinct regimes: *Squeezing*, *Dripping*, and *Jetting*. Increasing *Ca* promotes the transition from surface tension-dominated to viscous forcedominated regimes, with direct implications for the mechanism and size of the generated droplets.

The Weber number (*We*) expresses the ratio between inertial forces and surface tension:

$$We = \frac{\rho u^2 L}{\gamma} \tag{3}$$

Although negligible in most microfluidic flows, inertial effects become significant under high velocity conditions or near the droplet breakup point.

The Bond number (*Bo*) evaluates the relative importance of gravity compared to surface tension:

$$Bo = \frac{\Delta \rho g L^2}{\gamma} \tag{4}$$

where $\Delta\rho$ is the density difference between the phases. In microfluidic devices, *Bo* is generally very low ($Bo \ll 1$), indicating that gravitational effects can be neglected. Another two dimensionless numbers are also relevant: the viscosity ratio λ and the flow rate ratio φ between the dispersed and continuous phase fluids:

$$\lambda = \frac{\eta_d}{\eta_c} \quad \varphi = \frac{Q_d}{Q_c} \tag{5}$$

where η_d and η_c are the viscosities of the dispersed and continuous phases, respectively, and Q_d and Q_c are the volumetric flow rates of the two phases.

1.2. Droplet generation techniques

Droplet generation processes are a fundamental aspect of numerous microfluidic applications and can generally be classified into two main categories: passive and active methods. In so-called passive methods, droplet formation is governed exclusively by the intrinsic balance of fluid dynamic forces—capillary, viscous, and inertial—without the application of externally imposed energy fields. In this sense, they originate solely from the imposed pressure-driven motion and geometrical confinement, rather than from external actuation. Depending on the flow conditions, different regimes can be observed, including *squeezing*, *dripping*, *jetting*, *tip-streaming*, and *tip-multi-breaking* [43,44]. By contrast, active methods deliberately modulate droplet generation through external energy inputs—such as electric, magnetic, thermal or mechanical actuation—thereby exerting finer control over the breakup process and enabling the adjustment of key parameters including fluid velocities and material propertiest such as viscosity, interfacial tension, channel wettability, and fluid density [45].

1.2.1. Passive droplet generation

In passive systems, fluid flow is driven by external pumps, such as syringe or pressure pumps. These systems exploit intrinsic forces, such as hydrodynamic instabilities or capillary action, to facilitate droplet formation. Although passive methods are relatively simple and well-suited for compact chip designs, they generally provide less precise control over droplet size and generation frequency than their active counterparts. In passive systems, which are typically powered by external pumps, two immiscible fluids—the dispersed phase and the continuous phase—converge at a junction, where the immiscible interface undergoes deformation and eventual droplet breakup [43,46,47]. According to the complex geometrical design of the microchannel junction, the four most common microfluidic geometries used in passive droplet generation are a) co-flow, where both fluids flow in the same direction and the continuous phase surrounds and fragments the dispersed phase into droplets; b) cross-flow, where the continuous phase flows perpendicularly to the dispersed phase and induces its breakup by shear forces; c) flow-focusing, where the continuous phase compresses the dispersed phase within a narrow channel, leading to symmetric breakup and the formation of monodisperse droplets; d) step emulsification, where the dispersed phase flows through a shallow channel and abruptly enters a deeper reservoir, causing droplets to form due to a sudden reduction in confinement and capillary pressure, typically producing highly uniform droplets with minimal dependence on flow rate. Schematic illustrations of droplet generations with different microchannel geometries are shown in Fig. 3A, while a representative example of step emulsification is illustrated in Fig. 3B. In contrast to the shear-based mechanisms of the first three, step emulsification exploits a sudden change in channel depth to induce capillary pressure driven droplet breakup, yielding highly monodisperse droplets largely independent from flow rate.

1.2.1.1. Co-flow geometry. In the co-flow configuration, two immiscible phases flow in the same direction through a set of coaxial microchannels. The dispersed phase is introduced via an inner channel, while the continuous phase flows through an outer concentric channel, as illustrated in Fig. 3A(i) [20]. This concept, initially proposed by Umbanhowar et al. [48], can be implemented using either a quasi-two-dimensional planar device or a three-dimensional coaxial device. In these setups, the dispersed and continuous phases are aligned into parallel flows, creating a stable immiscible interface for droplet generation. The main parameter influencing droplet size in co-flow systems is the flow rate ratio (Q_d/Q_c), which determines whether the system operates in a dripping or jetting regime. In the dripping regime, droplet detachment occurs near the nozzle due to interfacial tension overcoming shear stress. In the jetting regime, a stable thread forms and breaks downstream due to Rayleigh–Plateau instabilities. In contrast to

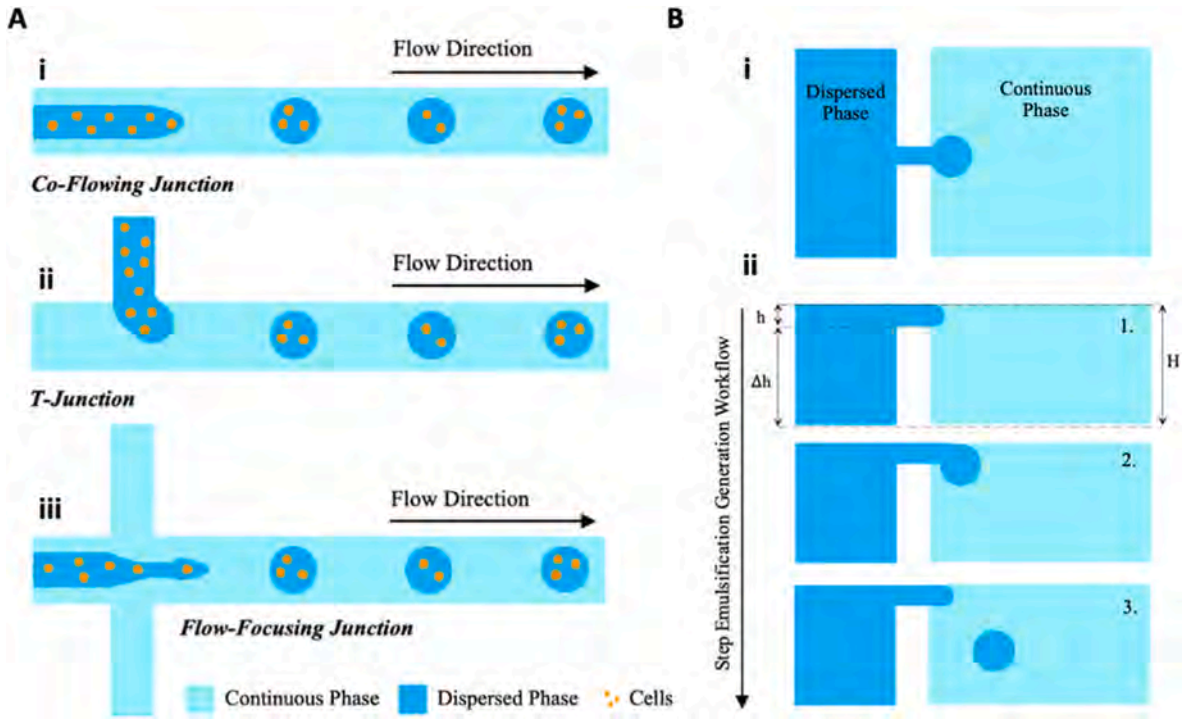

Fig. 3. (A) Schematics of the generation of cell-laden hydrogel microcapsules by droplet-based microfluidics. There are three basic types of microfluidic devices for cell encapsulation: (i) coaxial; (ii) T-junction; and (iii) flow-focusing junction. (B) Schematic representation of the step emulsification mechanism: (i) Top view of the microfluidic architecture, showing the dispersed phase flowing through a shallow nozzle into a deep reservoir filled with the continuous phase. (ii) Side view of the microfluidic architecture, showing cross-sectional sequence of droplet formation in two lateral directions [47].

other systems, such as flow-focusing geometries, co-flow configurations do not rely on geometric constriction but rather on viscous forces along the flow direction, making them particularly suitable for high-viscosity systems or when working with fragile biological samples. Channel dimensions—especially the inner capillary diameter—directly influence the minimal achievable droplet size. Moreover, co-flow devices exhibit a reduced dependence on channel surface wettability, making them robust in systems where surface treatment is challenging. Co-flow geometry has been widely employed for biocompatible emulsification, such as single-cell encapsulation, due to its low shear stress and gentle flow conditions. It is also particularly suitable for producing core–shell droplets, double emulsions, and monodisperse particles in pharmaceutical formulation, nutraceutical delivery, and materials chemistry.

1.2.1.2. Cross-flow geometry (T-junction). The cross-flow configuration is achieved through angled microchannels, where the continuous and dispersed phases meet at an angle between 0° and 180°. Among these, the T-junction configuration, where the phases intersect perpendicularly, is the most common and historically the first cross-flow geometry developed for droplet generation, as illustrated in Fig. 3A(ii) [20]. This setup induces a shear stress perpendicular to the interface between the two immiscible phases, generating nonlinearities and instabilities that promote droplet formation. T-junction geometries are particularly valued for their simplicity and ability to produce highly monodisperse droplets, with a coefficient of variation (CV) as low as 2 %. These features make them especially suitable for applications such as single-cell analysis, particularly in light of recent advancements that further improve droplet uniformity [49,50]. Xu et al. demonstrated that the hydrodynamic regime in a T-junction is influenced by interfacial instability, material wettability properties, viscous forces in the continuous phase, and the interfacial tension between the two immiscible fluids [51]. They developed an empirical equation to estimate droplet volume:

$$V_d(\mu L) = 0.024\left(\frac{Q_w}{Q_o}\right)^{-0.5} \tag{6}$$

where V_d is the droplet volume, Q_w is the flow rate of the aqueous phase, and Q_o is the flow rate of the oil phase. Furthermore, the droplet diameter D_d can be estimated as:

$$D_d = \frac{D_i}{C_a} \tag{7}$$

where D_i is the hydraulic diameter at the T-junction and Ca is the Capillary number. These relationships highlight that droplet size is positively correlated with the aqueous flow rate Q_w and the viscosity of the continuous phase.

1.2.1.3. Flow-focusing geometry. The flow-focusing geometry consists of three channels: a main channel and two symmetric side channels. These channels converge at a narrow constriction that connects to the downstream channel. In this region, the two immiscible phases flow coaxially, and the narrowing serves as a shear-focusing mechanism, promoting the formation of uniform droplets, as illustrated in Fig. 3A(iii) [20]. This configuration is particularly effective for producing relatively small droplets, with the droplet generation process closely tied to the dimensions of the narrow region. An advantage of this design is that the dispersed phase experiences only the driving force within the focused zone. The symmetry of the side channels ensures that forces from opposing directions cancel out, minimizing disturbances to encapsulated cells and enhancing droplet stability. The droplet size is primarily determined by the flow rate ratio of the two phases: higher continuous-phase velocity results in smaller, more rapidly generated droplets. In addition to the size of the focusing region, the liquid viscosity plays a significant role in influencing droplet formation and size. This makes flow-focusing geometries highly versatile and effective in achieving controlled droplet generation.

1.2.1.4. Step-emulsification. Step emulsification is a passive droplet generation mechanism that relies on capillary pressure differences induced by a geometric discontinuity—typically a sudden expansion in channel depth—to drive the spontaneous breakup of droplets [15,47]. In contrast to the shear-based methods such as T-junctions or flow focusing systems, step emulsification does not require high flow rates or lateral compression from a continuous phase. Instead, droplet formation is governed primarily by interfacial tension and the geometry of the microchannel. The physical basis of step emulsification is well described by a quasi-static model, in which the interface of the dispersed phase evolves slowly enough that the system remains in near-equilibrium during most of the droplet formation process [52]. According to this model, as the dispersed phase thread exits the shallow channel and enters the deeper reservoir, a bulb begins to form downstream of the step. The mean curvature of the interface decreases as the bulb grows, requiring a corresponding adjustment in the curvature of the upstream thread to maintain pressure balance across the interface, as dictated by the Young–Laplace equation:

$$\gamma\kappa = p_i - p_o \tag{8}$$

where γ is the interfacial tension between the inner (dispersed) and outer (continuous) phases, κ the mean curvature of the interface—defined as the sum of the main curvatures along two orthogonal directions, p_i is the pressure within the inner phase, and p_o is the pressure exerted by the outer phase. When the upstream thread can no longer compensate for the decreasing curvature of the bulb, a necking region forms at the transition. As the neck narrows, its cross-sectional diameter decreases progressively; once it approaches the nozzle height h, the critical geometric threshold, the quasi-static equilibrium breaks down, and droplet detachment is triggered by the Rayleigh–Plateau instability [53]. This mechanism, illustrated schematically in 3B (i, ii), results in the spontaneous formation of monodisperse droplets with a size primarily dictated by the nozzle height, and largely independent of flow rate within the dripping regime. This mechanism makes step emulsification particularly attractive for applications requiring high droplet monodispersity with minimal external control. Moreover, the absence of strong shear forces enhances its compatibility with fragile biological samples, such as live cells or enzymes, and facilitates integration in parallelized or portable platforms.

1.2.2. Droplet active generation

Unlike passive methods, active techniques enable more flexible and dynamic control over droplet formation by introducing external energy inputs into the system. This added control allows for precise tuning of droplet size, frequency, and timing, enabling on-demand production that is particularly advantageous in applications requiring high responsiveness or droplet uniformity. Active techniques are generally categorized according to the type of energy applied—namely electric [54], magnetic [55,56], thermal [57] or mechanical (such as piezoelectric) stimuli [58,59]. The balance of interfacial forces is altered through two main strategies: the introduction of additional forces (electric, magnetic, thermal), and the modulation of viscous, inertial, and capillary forces by varying the flow velocity or the physical properties of the materials involved. For a more detailed discussion, readers are referred to a brilliant review by Chong et al. [45].

1.2.2.1. Electrical method. In electrical methods Fig. 4A, a direct current (DC) electric field can be applied across microchannel-integrated electrodes to manipulate the droplet interface. The induced electrostatic forces alter interfacial tension and promote droplet pinch-off at controlled rates. This principle has been applied in flow-focusing geometries using ITO electrodes, where increasing the voltage results in smaller droplets [54]. Although effective, DC-based systems may suffer from issues such as electrode degradation or the production of highly charged droplets, which can be problematic for biological samples.

1.2.2.2. Magnetic method. Magnetic actuation Fig. 4B leverages external magnets to control the dynamics of ferrofluid droplets. By positioning a magnet either upstream or downstream of the droplet generation site, it is possible to modulate the breakup process and tailor droplet size [56]. This strategy benefits from the contactless nature of magnetic fields but is limited to magnetically responsive fluids.

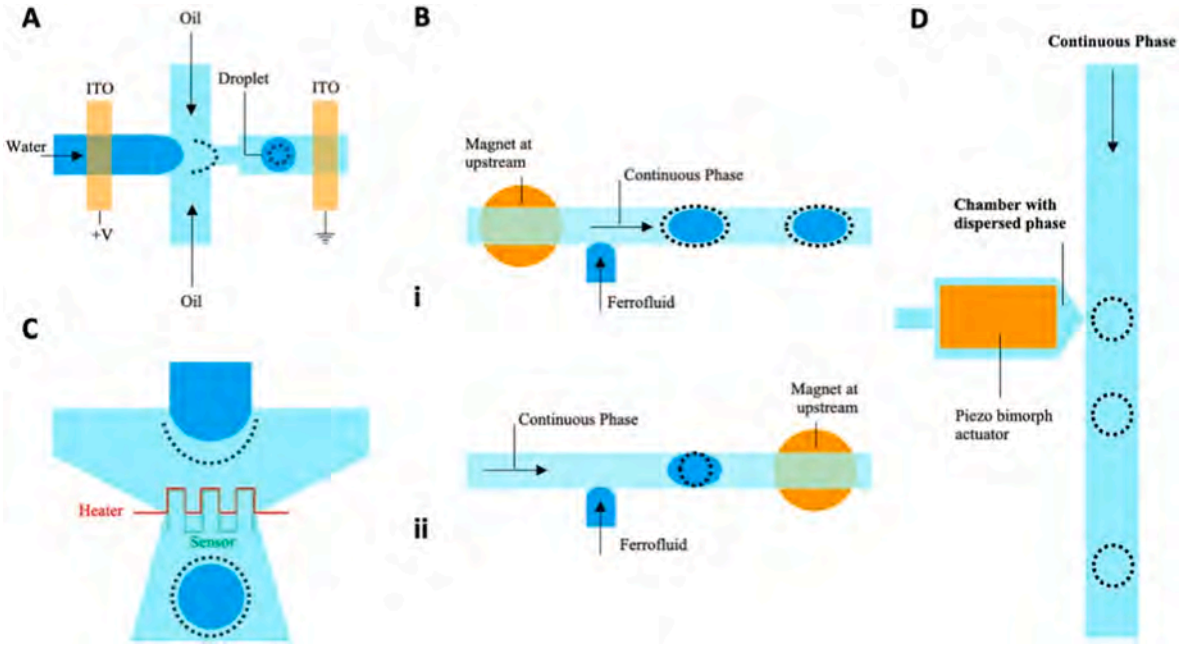

Fig. 4. Droplet generation by active methods. (A) Droplet generation by applying a direct current voltage. (B) T-junction device with ferrofluid and magnet at (i) the upstream position and (ii) the downstream position. (C) Schematic representation of the microfluidic device designed to study the temperature dependence of the droplet formation process. (D) Microfluidic chip for on-demand droplet dispensing: a piezoelectric actuator releases aqueous droplets into a vertical channel with immiscible fluid [47].

1.2.2.3. Thermal method. Thermal control Fig. 4C involves modulating local temperature to affect fluid viscosity and interfacial tension. This can be achieved via integrated microheaters or laser-induced heating. For instance, increasing temperature generally lowers interfacial tension, facilitating the formation of larger or smaller droplets depending on flow conditions [57]. Thermal approaches offer precise local control but may require careful regulation to avoid undesirable thermal gradients or damage to thermosensitive materials.

1.2.2.4. Mechanical method (piezoelectric). Piezoelectric actuation Fig. 4D enables high-speed, on-demand droplet dispensing through the mechanical deformation of piezoelectric elements. When a voltage pulse is applied, the actuator rapidly deforms, ejecting a droplet into the continuous phase. This method is particularly suited to applications demanding high throughput and fine control, such as digital printing or rapid diagnostics [58].

Together, these active strategies expand the functional versatility of droplet-based microfluidics, offering precise control over droplet production in ways not achievable through purely passive means. Their integration into microfluidic platforms has opened the door to increasingly sophisticated operations in diagnostics, drug delivery, and synthetic biology. However, active techniques often come with trade-offs, including increased system complexity, higher fabrication costs, and challenges in integration and long-term reliability. These factors can hinder their widespread adoption, particularly in low-resource or portable settings. A detailed comparative assessment of passive and active droplet generation methods—including performance, cost, scalability, and biocompatibility—is provided in Fergola et al. [47]. This review also includes a visual summary that highlights the strengths and limitations of each strategy, offering guidance for method selection based on specific application requirements.

2. Structured materials engineered through droplet-based microfluidics

In microfluidic devices, DBM represents one of the most promising platforms for the controlled generation of micro- and nanoparticles, with significant applications in drug delivery and cell encapsulation. As will be further discussed in the following sections, this technology enables the production of engineered microcarriers with advanced structural and functional properties, starting from natural or synthetic materials. DBM offers a versatile and efficient alternative to traditional particle fabrication methods, combining precise control over particle size and uniformity with the ability to encapsulate sensitive compounds or living cells. Its capacity for high-throughput production, compatibility with biomaterials crosslinkable under physiological conditions, and scalability further establish DBM as a pivotal technology in tissue engineering and regenerative medicine. The following sections will analyze the main types of carriers produced using the DBM approach, ranging from the nanoscale—with the production of polymeric nanoparticles—to the microscale, focusing on solid-structured microparticles, microcapsules, and microfibers. Particular attention will be given to their morphological properties, material composition, and the release strategies employed. Application examples will also be presented in which carriers were designed to achieve specific functionalities, such as sustained release of active agents, selective targeting, or support for three-dimensional cell proliferation. This dual approach—targeting both controlled drug delivery and cell encapsulation—confirms the central role of DBM in the design of personalized biomedical platforms.

2.1. Polymer nanoparticles

Polymers are widely used for drug delivery systems due to their biocompatibility, biodegradability, and ease of chemical modification, which allow for efficient design and preparation of carriers able to deliver therapeutic agents to target tissues [60]. Among polymeric nanocarriers, nanoparticles represent a broad structural class, encompassing architectures specifically designed to encapsulate both hydrophilic and hydrophobic drugs and widely applied in cancer therapy. Within this overarching category, three main subtypes are typically distinguished — nanospheres, nanocapsules, and nanogels — each characterized by distinct structural and release properties (see Fig. 5) [61,62]. Conventional methods for nanoparticle (NP) production, although widely used and well-established, present several limitations: they require large volumes of costly reagents, often result in polydisperse particles, offer limited potential for co-encapsulation of multiple therapeutic agents, and exhibit poor batch-to-batch reproducibility. Moreover, the challenge of ensuring morphological and physicochemical uniformity hinders large-scale clinical production and slows down the translation of nanomedicines. In this context, microfluidic systems—and particularly DBM—have emerged as a promising solution. Microfluidics offers the possibility to simplify the design of novel NP-based systems with tunable physicochemical properties such as size, size distribution, and morphology, while ensuring high batch-to-batch reproducibility and improved therapeutic efficacy in both *in vitro* and in vivo settings.

2.1.1. Nanospheres

Nanospheres are homogeneous matrix systems in which a dispersed or dissolved therapeutic compound is adsorbed on the surface or trapped within the polymer matrix structure throughout the solid sphere (Fig. 5A) [63]. These systems are characterized by sustained and

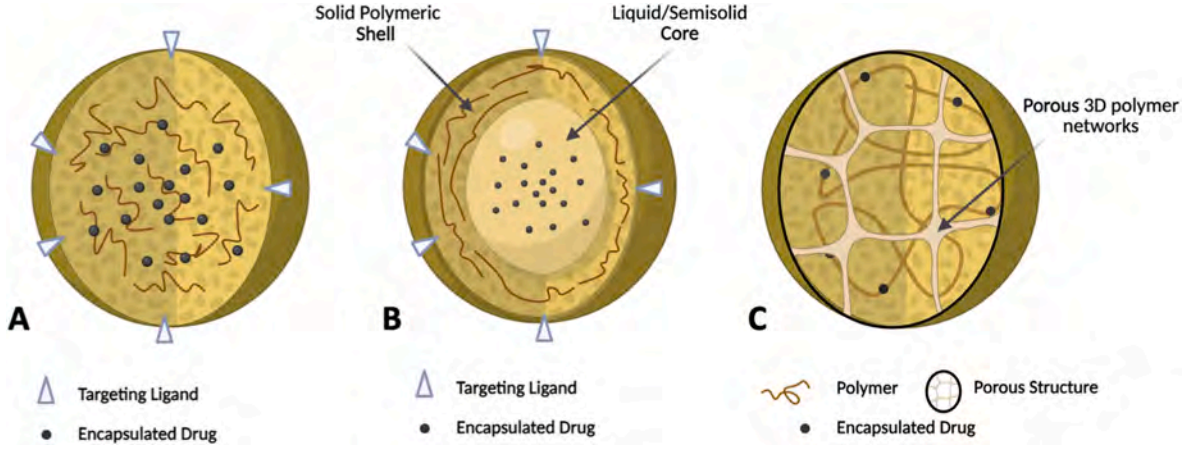

Fig. 5. (A) Typical schematic structure of a polymeric nanosphere. (B) Typical schematic structure of a polymeric nanocapsule. (C) Schematic structures of nanogels obtained by self-assembly process with a nucleation site.

controllable drug release and high drug loading, both for hydrophilic and poorly water-soluble drugs.

A notable example of nanosphere generation using microfluidic technology was reported by Karnik et al., who developed a microfluidic platform for the controlled production of solid polymeric nanoparticles [64]. The platform relies on a flow-focusing junction device, enabling fine modulation of key parameters such as flow rates, polymer composition, and concentration. This configuration allowed optimization of particle size, reduction of polydispersity, and enhanced control over the final physicochemical properties. The authors used a (poly(lactic-co--glycolic acid)-polyethylene glycol) (PLGA-PEG) block copolymer, dissolved in acetonitrile as the organic/dispersed phase, with water introduced laterally as an antisolvent. The rapid mixing achieved within the microchannel promotes on-chip nanoprecipitation, leading to the formation of uniform nanospheres. This approach enabled the production of particles ranging from 30 to 100 nm in diameter, characterized by high monodispersity and reproducibility, overcoming the limitations of conventional batch synthesis. The use of high molecular weight PLGA further increased particle stability and enhanced the encapsulation efficiency of docetaxel. The resulting system exhibited a slow and controlled drug release profile, with potential therapeutic benefits in terms of sustained delivery and reduced side effects.

2.1.2. Nanocapsules

Nanocapsules are characterized by a reservoir form, in which a solid polymer shell surrounds a core that is either liquid or semisolid (Fig. 5B) [65]. Nanocapsules with a lipid core allow a high payload of a liposoluble drug, whereas nanocapsules with an aqueous core are able to encapsulate water soluble compounds. Furthermore, nanocapsules can also carry the active substance on their surfaces or absorbed in the polymeric shell [66,67].

Yang et al. proposed an innovative strategy for synthesizing core–-shell nanoparticles via microfluidic-guided self-assembly [68]. Specifically, the process occurs within a microfluidic device, where rapid mixing and solvent exchange between ethanol and water induce the co-precipitation and self-assembly of a mixture containing drug, oil, polymer, and a functionalized copolymer (PEG-modified). This mechanism, driven by interfacial energy minimization, leads to the formation of core–shell nanocapsules with tunable surface functionalities. The resulting poly(ϵ-caprolactone)-Poly(ethylene glycol)- Folic Acid (PCL-PEG-FA) nanocapsules, resulting in a polymeric shell functionalized with folic acid, exhibited high encapsulation efficiency, excellent loading capacity, and outstanding performance in anticancer drug delivery, both *in vitro* and in vivo. Tumor targeting was achieved through specific interaction between the surface functional group of the nanocapsules and the corresponding receptor expressed on the cancer cell membrane.

2.1.3. Nanogels

Nanogels are soft, highly hydrated, three-dimensional polymer networks capable of absorbing large amounts of water while maintaining structural integrity (Fig. 5C). Their porous structure allows the encapsulation of various bioactive molecules, including proteins, nucleic acids, or chemotherapeutics. Nanogels exhibit excellent biocompatibility and can respond to external stimuli—such as pH, temperature, or enzymatic activity—making them ideal for stimuli-responsive and site-specific drug delivery. Their tunable size, surface functionality, and deformability enhance tissue penetration and circulation time in vivo.

Giannitelli et al. [69] developed an advanced microfluidic platform based on a flow-focusing geometry integrated with a pneumatic microactuator capable of modulating the junction orifice width in real-time. This design allowed highly precise control over mixing and flow regimes, directly impacting nanogel size and monodispersity. The system enabled the generation of highly uniform nanogels with tunable diameters ranging from 92 to 190 nm and extremely low polydispersity (0.015)—results difficult to achieve with conventional batch methods. The nanogels, based on hyaluronic acid (HA) and linear polyethyleneimine (LPEI), were loaded with doxorubicin (DOX) through electrostatic interactions and entrapment within the crosslinked network, achieving high encapsulation efficiencies and controlled drug release. The platform was validated *in vitro* on ovarian carcinoma models, showing significantly higher anticancer activity compared to free DOX, even at sublethal doses. This result is attributed to the combination of active targeting (mediated by HA toward tumor CD44 receptors) and the sustained release profile provided by the nanogel matrix. Overall, the work proposes a highly tunable microfluidic strategy for the production of functional and clinically relevant nanogels, opening new perspectives in personalized drug delivery and precision oncology.

2.2. Microparticles

One of the main strengths of droplet-based microfluidics (DBM) lies in the ability to precisely design both the composition and morphology of droplets, enabling the production of highly functionalized polymeric microparticles [70,71]. These structures are widely used as drug encapsulation and controlled release carriers, but they also work incredibly well as three-dimensional cell culture microenvironments. The dual application of microparticles — as drug carriers and as microreactors for cells — is closely linked to their morphological versatility, the ability to regulate the permeability of the polymer matrix, and their compatibility with various polymerization strategies. Because of their therapeutic relevance in complex clinical scenarios, such as combination therapy or targeted delivery, their ability to adapt to molecules with different physicochemical properties, and the potential to control release profiles, the focus of this section will primarily be on microcarriers designed for the delivery of active compounds. However, it will also be highlighted how the same particles, when properly engineered, can serve as support systems for *in vitro* cell growth, offering a scalable and replicable platform for 3D tissue models. The main production mechanism as well as the morphological and structural characteristics of solid-structured polymeric microparticles are examined in this part of the paper. Attention will also be devoted to the generation of multicompartment microparticles, characterized by distinct functional compartments not achievable through conventional approaches, and finally to core–shell microparticles, designed for the selective encapsulation of hydrophilic or hydrophobic compounds, featuring high loading efficiency and controlled release.

2.2.1. Solid polymeric microparticles

Solid-structured polymeric microparticles represent one of the most extensively studied types in the context of droplet-based microfluidics production [72]. In these systems, the solidified polymer phase allows the fabrication of particles characterized by remarkable morphological stability, uniform size, and controllable surface properties. By providing circular, monodisperse particles with highly repeatable morphologies, microfluidic devices help to overcome the drawbacks of traditional synthesis techniques, such as batch-to-batch size variability [73]. In addition to achieving precisely regular spherical shapes, other polymerization techniques and appropriate modulation of droplets confinement within the microchannels can produce more complicated morphologies, including rods, ellipsoids, and asymmetric structures. Depending on the internal distribution of materials, two main morphological categories can be distinguished: homogeneous solid microparticles and heterogeneous-structured microparticles, each exhibiting specific mechanical and diffusional properties.

2.2.1.1. Homogeneous-structured microparticles. Homogeneous-structured microparticles are characterized by a continuous and uniform polymeric matrix, within which any functional agent can be evenly distributed throughout the entire particle volume. This configuration

results in a solid, compact morphology with no internal discontinuities, making these particles particularly suitable for applications requiring high structural stability, molecular cargo protection, or controlled release. For instance, such structures are frequently employed for the delivery of high molecular weight compounds, such as protein-based drugs. The ability to produce both spherical and non-spherical particles by the controlled deformation of droplets within microchannels is one of the most significant morphological properties. The creation of particles with narrowly defined diameters, based on the volume of the precursor droplets, is further facilitated by the use of these microfluidic systems. In some cases, the solid structure can take the form of a crosslinked and permeable hydrogel, particularly suited for the release of bioactive macromolecules. By adjusting the size, content, and droplet formation dynamics, it is simple to modify the encapsulation efficiency and the final particle properties, providing exceptional design flexibility. Water-in-oil (W/O) or oil-in-water (O/W) emulsions are the primary methods for producing homogeneous-structured microparticles. Using water-in-oil (W/O) droplets as templates, it is possible to produce monodisperse microparticles with crosslinked hydrogel networks by dissolving monomers or water-soluble polymers in the aqueous phase and subsequently inducing polymerization or crosslinking [72,74]. On the other hand, starting from oil-in-water (O/W) droplets, lipophilic polymers dissolved in the oil phase can be solidified via polymerization or solvent evaporation. The work of Ekanem et al. made a substantial contribution to the development of solid, homogeneous structured microparticles using DBM [75]. In this context, the authors created oil-in-water (O/W) emulsions using Polylactic Acid (PLA) or PLGA dissolved in volatile organic solvents using axisymmetric flow-focusing junction microfluidic devices. Monodisperse microparticles (4–30 μm) with uniform interior structures were then produced as a result of solvent evaporation. A schematic illustration is depicted in Fig. 6A. In particular, the surface morphology and internal microstructure of the particles were modulated by the addition of nanofillers (nanoclay) and porogens, resulting in reduced porosity and rough surfaces, respectively. This approach enabled the fabrication of solid, morphologically uniform particles where the active compound could be uniformly distributed within the polymeric network. This study thus confirms the effectiveness and versatility of microfluidic platforms in generating homogeneous solid microcarriers functionalizable for advanced drug delivery applications. Fabrication mechanisms for solid, homogeneous-structured microparticles are not limited to microsphere generation. Thanks to the design flexibility of microfluidic devices, it is possible to obtain emulsified templates with alternative shapes. For instance, controlled deformation of the droplet morphology can be achieved by employing microchannels that are smaller than the droplet size. Subsequent in situ polymerization of the deformed droplets "fixes" their shape, resulting in non-spherical solid microparticles [76]. An interesting example is the production of toroidal microparticles achieved through asymmetric polymer solidification within a microchannel (see Fig. 6B) [77]. In this approach, droplets containing the polymer in an organic solvent flow through an environment where the solvent diffuses anisotropically into the continuous phase, leading to preferential solidification at the droplet periphery. This mechanism enables the formation of complex-shaped particles with homogeneous internal morphology, demonstrating how engineered microfluidics can overcome the natural tendency of droplets to form spheres, thus enabling the synthesis of anisotropic solid structures.

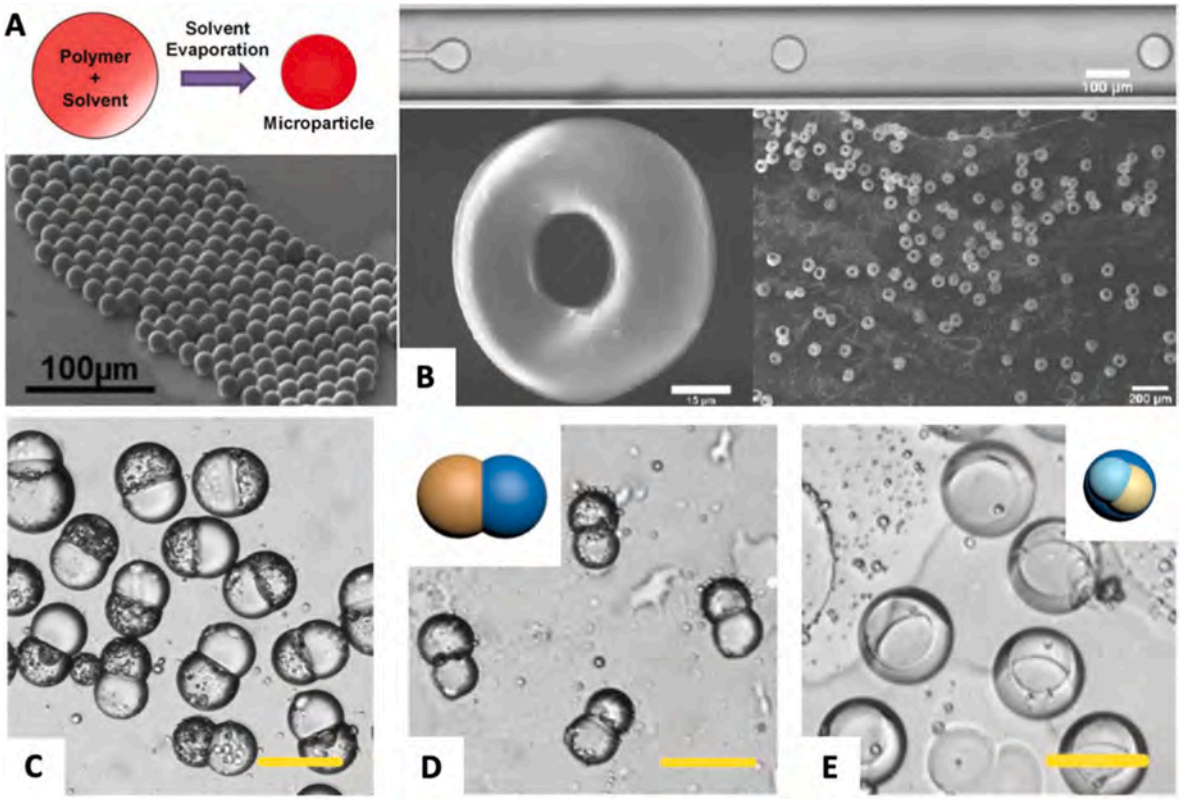

Fig. 6. Microfluidic generation of solid microparticles with homogeneous and heterogeneous internal structures. (A) Monodisperse spherical microparticles produced via solvent evaporation of PLA/PLGA solutions; Scanning Electron Microscopy (SEM) image shows particles with a mean diameter of 16 μm [75]. (B) Toroidal microparticles fabricated through anisotropic polymer solidification within a confined microchannel. Images show droplet formation (top), SEM of a single toroid and population view (bottom) [77]. (C–E) Janus and multicompartment particles: (C) phase-separated particles composed of lipid and pH-sensitive polymer; (D) Janus particles with clearly defined hemispheres; (E) multicompartment particles combining fatty acids and polymers for thermally and pH-triggered sequential drug release (All scale bars: 100 μm) [78].

2.2.1.2. Heterogeneous-structured microparticles. Heterogeneous-structured microparticles are characterized by a non-uniform distribution of materials within them, featuring compartmentalized structures or asymmetric domains [79–81]. Thanks to the flexibility in microchannel design and the precision in flow manipulation, droplet-based microfluidics (DBM) enables the generation of biphasic droplets through parallel flows. This approach allows the formation of emulsified templates that give rise to Janus microparticles and, more generally, heterogeneous structures, via phase separation of two immiscible polymers confined within droplets. The type of surfactant used and the polymer pair employed can strongly influence the final shape of the solid particles since they affect the interfacial behavior during the solidification process. From a morphological and structural perspective, these particles exhibit a clear phase separation within their geometry, enabling extremely precise spatial distribution of the cargo. Such configurations allow, for example, the localization of drugs or fluorescent probes in well-defined regions of the particle, and the resulting anisotropic morphology can be exploited to promote selective interaction with target tissues or to achieve differential release. Furthermore, it is possible to optimise crucial factors, such as surface functionalization, hydrophilicity, and biodegradability, by combining materials with different qualities into a single particle. A significant example is again provided by the study of Ekanem et al., in which Janus particles were produced via phase separation induced by solvent evaporation within droplets containing an organic mixture of PLA/PCL (30/70 or 70/30 v/v) dissolved in dichloromethane [75]. The process culminated with the formation of solid microparticles comprising two distinct hemispheres, corresponding to the two polymeric components. Each hemisphere exhibited different physicochemical properties, with the potential to be useful for multi-phase controlled release. Another significant contribution is offered by the study of Feng et al., in which Janus microparticles were generated by combining a pH-sensitive polymer phase with a thermoresponsive lipid phase [78]. Phase separation occurred during solvent evaporation, resulting in two functionally distinct hemispheres: the lipid component melts at physiological temperature, enabling immediate release of the encapsulated drug, whereas the polymeric domain degrades in acidic environments typical of tumor tissues, releasing the therapeutic cargo selectively. Morphological images shown in Fig. 6C, D highlight the clear separation between the two phases. This work also demonstrated the possibility of extending particle functionality by introducing additional materials, such as 1-tetradecanol, 1-hexadecanol, and lauric acid, enabling programmable and multimodal release strategies, as shown in Fig. 6E. Finally, Liu et al. developed a microfluidic strategy based on external gelation for the production of Janus microparticles composed of calcium alginate hydrogel, characterized by two distinct and well-defined compartments [82]. The method enabled the generation of monodisperse microparticles with diameters ranging from 148 to 179 μm (CV < 4 %), where the volumetric proportion of the two domains could be modulated by varying the flow rate ratio of the dispersed phases. The resulting particles demonstrated clear morphological and structural separation between compartments, and could also be functionalized with magnetic and fluorescent agents, rendering them easily manipulable by using external magnetic fields. Furthermore, it was demonstrated that such Janus microparticles are suitable for cell encapsulation, ensuring good cell viability and opening new perspectives for applications in physical, biochemical, and biomedical fields. Overall, these studies confirm that microfluidic technology represents an ideal platform for the design and production of morpho-functional heterogeneous microparticles, particularly suitable for advanced applications in targeted drug delivery, selective stimulation, and multiphase cell encapsulation systems.

2.3. Core-shell microcapsules

Microcapsules, commonly referred to as core–shell structures, consist of a central core surrounded by an outer shell, typically composed of film-forming materials. These particles, with diameters ranging from the micrometer to the nanometer scale, exhibit high dimensional uniformity and structural consistency. In this context, droplet-based microfluidics plays a pivotal role, enabling the precise generation of multiphase droplets — such as double or multiple emulsions — and providing an ideal platform for the fabrication of core–shell microstructures. Moreover, this technology facilitates the formation of highly monodisperse capsules with tunable morphological characteristics, including size, composition, and shell thickness, through the accurate manipulation of multiphase flow dynamics within microchannels. Compared to solid matrix microparticles, core–shell architectures offer an enhanced degree of compartmentalization, allowing for the combination of distinct materials in the core and shell regions. For example, the use of an aqueous or oily core enables the selective encapsulation of hydrophilic or hydrophobic compounds, respectively, Fig. 7A and B, while the shell composition can be engineered to tailor mechanical protection, permeability, or responsiveness to environmental stimuli.

These structural features directly influence application performance. For instance, Galogahi et al. fabricated spherical microparticles using water-in-oil-in-water W/O/W double emulsions generated in a surfactant-free flow-focusing microfluidic device (see Fig. 8A) [83]. After photopolymerization, they obtained core–shell particles with a central liquid core isolated by a regular polymeric shell. The study demonstrated a direct correlation between shell thickness and particle rupture strength, highlighting the importance of morphological design in defining mechanical properties, with remarkable implications for drug delivery applications. Similarly, in the work carried out by Yang et al., microcapsules with oily cores were designed by encapsulating drugs both in free form and within PLGA nanoparticles inside a lipophilic core enclosed by a crosslinked chitosan shell [84]. The particles, exhibiting spherical morphology and well-defined core–shell structures, showed a sequential pH-responsive release: the acidic environment induced shell degradation, allowing rapid release of the oily content, followed by prolonged release from the polymeric nanoparticles dispersed in the core (see Fig. 8B). In this case, the functional distinction between the internal and external compartments directly results from the tailored core–shell architecture, once again highlighting the structural value of emulsified capsules in defining therapeutic performance. Beyond bioactive compound delivery, core–shell structures also show great potential as microcarriers for cell encapsulation. Wang et al. fabricated core–shell microgels composed of a Gelatin-Methacryloyl (GelMA) shell and a methylcellulose (MC) core using a droplet microfluidic device [85]. These microgels allowed for the encapsulation of hepatic cells, maintaining high viability over a period of 15 days, and supported coculture with endothelial cells for tissue engineering purposes. Time-lapse imaging and live/dead assays confirmed the structural and biological stability of the construct (Fig. 8C), underscoring the adaptability of core–shell formats for regenerative medicine applications.

2.4. Microfibers

The same geometries employed in DBM for the generation of microcarriers are also applied in the production of microfibers, leveraging the intrinsic ability of these platforms to operate with high precision at the microscale. Specifically, by carefully adjusting flow rates and operating conditions, it is possible to access the jetting regime — a mode that, unlike the dripping regime (characterized by the formation of discrete droplets), enables the generation of continuous, elongated flows that can subsequently be solidified into fiber form. Among the most commonly used architectures for this purpose are flowfocusing and multibarrier configurations, which enable the focusing of a central stream — typically composed of a polymeric or bioactive solution — by means of high-velocity coaxial flows. Upon reaching an appropriate capillary number, a stable jet is triggered, which can be

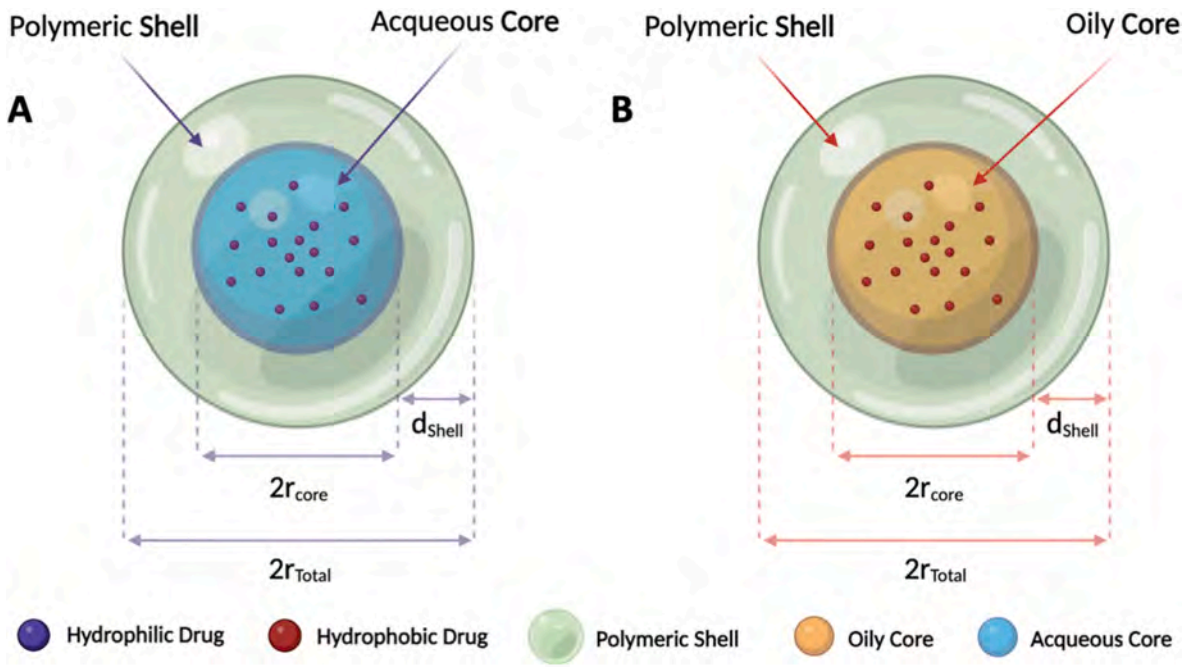

Fig. 7. Schematic representation of core–shell microparticles produced by DBM: (A) an aqueous-core particle suited for loading hydrophilic drugs (purple dots) and (B) an oil-core particle suited for hydrophobic drugs (red dots). Geometric parameters: r_{core} = radius of the inner core; d_{Shell} = thickness of the polymeric shell; r_{Total} = overall particle radius (from center to the outer shell surface), with: $r_{Total} = r_{core} + d_{Shell}$. Dashed double-headed arrows indicate the corresponding diameters ($2r_{core}$ and $2r_{Total}$). Color code: green, polymeric shell; blue, aqueous core; ochre, oily core. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

solidified through ionic crosslinking, photopolymerization, or solvent evaporation. This approach enables the fabrication of microfibers with controlled diameters, uniform morphology, and tunable composition, offering a refined and scalable alternative to conventional techniques such as electrospinning. Furthermore, many microfluidic technologies developed in this field allow for the production of microfibers with core–shell or multicompartmental structures, which are particularly suited for applications in controlled drug release or cell encapsulation, due to the ability to physically separate the functional content from the structural matrix. A notable example is offered by Cheng et al., who developed a multibarrel microfluidic device capable of continuously producing multicellular microfibers with tunable morphology and biochemical composition [86]. By co-injecting laminar flows of alginate and bioactive hydrogels such as GelMA or ECM-based solutions—each potentially laden with cells—they generated stable, crosslinkable jets that solidified via ionic gelation. This approach enabled the fabrication of hollow, layered, or compartmentalized fibers, suitable for use as biomimetic scaffolds in tissue engineering. As demonstrated with GelMA fibers seeded with Human Umbilical Vein Endothelial Cells (HUVECs), these structures can support vascular morphogenesis and allow for spatially controlled co-culture strategies (see Fig. 9).

Complementing this, a recent study by Guimarães et al. demonstrated the use of a three-dimensional flow-focusing microfluidic chip to obtain a continuous flow of hydrogel precursors, which self-organize according to relative viscosity and applied pressures [87]. The system allows for the production of microfibers with multicompartmental structures and unconventional shapes — including flat "ribbon-like" fibers, vascular core–shell fibers, and fibers integrated with stem cells and hydrophobic pro-differentiating molecules. This approach represents an extremely flexible platform for the biofabrication of complex three-dimensional models and the functional reproduction of biological architectures such as stratified tissues or tissue-like microenvironments. Taken together, the previous studies highlight the application potential of DBM geometries operating in the jetting regime not only for the structural control of microfibers but also for the advanced functionalization of the resulting systems, making them promising tools for regenerative medicine, experimental oncology, and tissue modeling.

3. Biomaterials used

To encapsulate bioactive molecules, drugs or cells, a 3D structure, e.g. scaffold, is required [88,89]. In particular, for cells encapsulation, several requirements must be met, such as biocompatibility, biodegradability, non-immunogenicity, permeability to oxygen and metabolites, and sufficient mechanical properties [90]. Table 2 provides synthetically specific biomaterials classes advantages and limitation. Additionally, Fig. 10 provides a timeline of the milestones reched in the field of DBM for biomaterial fabrication.

3.1. Solid polymers

For drug delivery applications, solid polymeric materials such as PLGA and PCL have been extensively used in literature [91,92]. Solid polymers applications are limited by their hydrophobicity, leading to in capacity of forming hydrogels like most natural polymers [93]. The solvents used in the manufacturing of solid polymers droplets are cytotoxic, and emulsion solvent evaporation or nanoprecipitation are not compatible with live cell encapsulation [94]. The main application of this class of materials is the synthesis of microparticles, microcapsules, or nanoparticles for drug delivery or imaging [94]. However, some strategies have been used to combine solid polymers with biocompatible hydrogels [95]. Table 3 resumes various studies involving solid polymers in DBM.

3.1.1. Poly lactic-co-glycolic acid (PLGA)

PLGA is a readily available copolymer approved by FDA for biomedical use. PLGA is biodegradable through hydrolysis, releasing lactate, that may be harmful to specific tissue as it causes acidification of the surrounding environment [93]. PLGA is extremely tunable by varying the ratio of Lactic Acid (LA) and Glycolic Acid (GA). Earliest

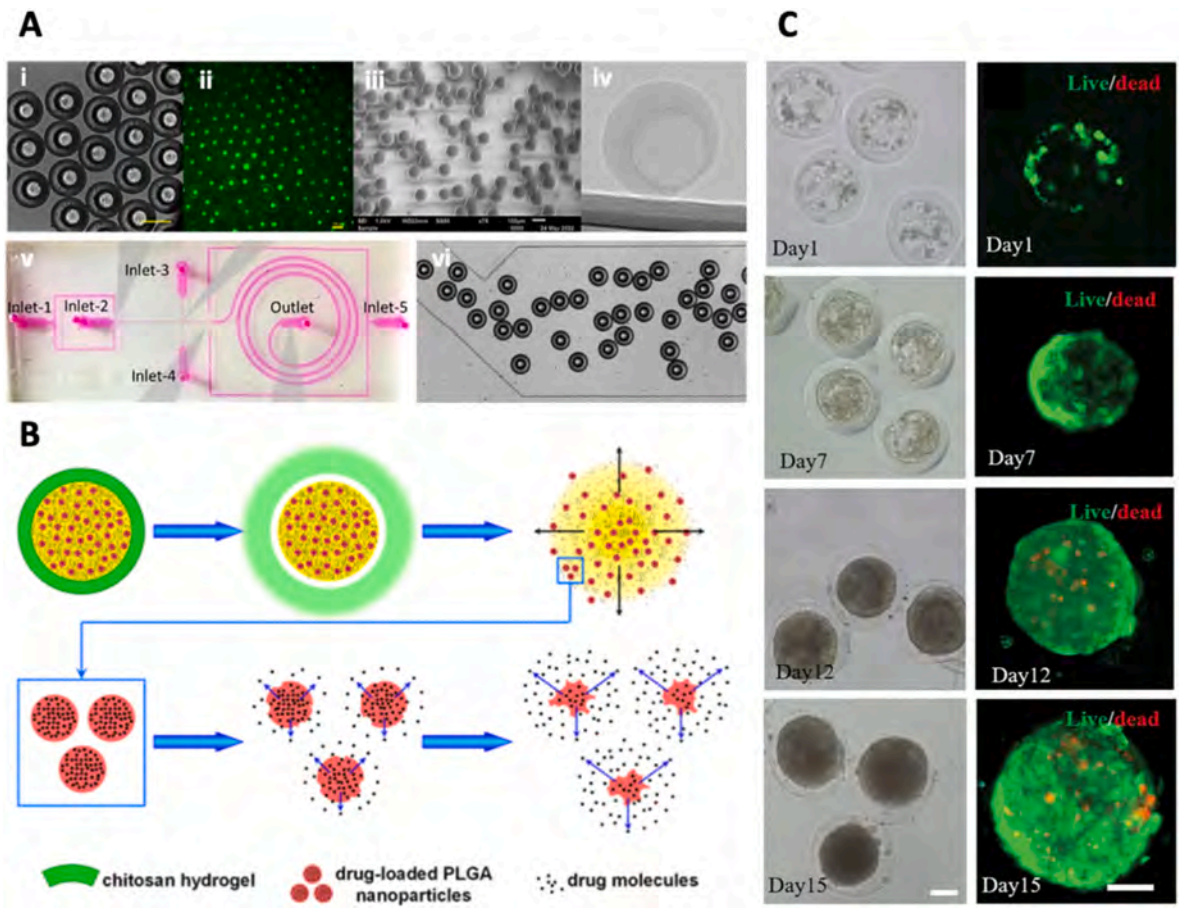

Fig. 8. Core–shell microcapsules generated via droplet-based microfluidics for drug delivery and cell encapsulation. (A) Fabrication process of spherical core-shell structure microparticles using W/O/W double emulsions: (i) Bright-field image of core–shell droplets with aqueous cores; (ii) fluorescence image of core–shell droplets; (iii) SEM image of cured solid microparticles; (iv) X-ray image of a representative particle; (v) fiveinlet microfluidic device used for droplet generation; (vi) overview of collected core–shell microparticles (All scale bars: 100 μm) [83]. (B) Schematic representation of the pHtriggered sequential release mechanism from core–shell chitosan microcapsules encapsulating both free drug and PLGA nanoparticles. Rapid chitosan degradation allows burst release, followed by sustained release via PLGA degradation [84]. (C) Bright-field and fluorescence live/dead imaging of hepatic cells encapsulated within GelMA-MC microgels at various time points (Day 1–15), confirming long-term viability and structural integrity of the core–shell construct for tissue engineering applications (Scale bars: 100 μm) [85].

report of PLGA in microfluidics includes the production of microfibers [96] and nanoparticles [64]. PLGA microfibers could be tuned in diameters through flow rate variations, with a controlled morphology providing a dense shell and porous core and have been preliminary used for cell orientation studies [96]. Nanoparticles produced by Farokhzad et al. demonstrated better control over their physicochemical properties compared to traditional bulk synthesis [64]. Higher control on the microparticles morphologies was performed in 2015 by Ekanem et al., obtaining solid polymer particles, nanoclay embedded particles, golf-ball like particles and hemisferical particles [75] (See Fig. 11B). More advanced drug-loaded nanoparticle systems were produced for the encapsulation of DOX and Tamoxifen (TAM) by Xu et al. in 2017 reaching encapsulation efficiencies as high as 88 % [97]. In 2024, Udepurkar et al. developed a chip for the production of PLGA nanoparticles integrating an ultrasonic element in the microfluidic device, allowing the researcher to obtain PLGA nanoparticles polydispersity index (PDI) < 0.3 with a size between 115 and 150 nm [98] (See Fig. 11C). Currently, semi-automated PLGA microparticle stations are being marketed (e.g. Fluigent's RayDrop droplet generator), For more in-depth analysis of PLGA uses in microfluidics, specialized reviews can be found in the literature [99].

3.1.2. Polycaprolactone (PCL)

PCL is a biodegradable semicrystalline polymer approved by FDA for biomedical use. Its main advantage with respect to PLA is that PCL does not generate acidic microenvironment [93], moreover, degradation time of PCL is usually longer than PLGA [100]. In 2009, Yang et al. successfully entrapped anticancer drug, fluorescent and superparamagnetic nanoparticles inside PCL microcapsules, using an oil-in-water emulsion in a microchannel cross-junction [101]. Vladisavljevic' et al. produced monodisperse biodegradable porous microparticles using a flow focusing device via solvent evaporation, combining experimental results and numerical modeling [102]. In 2016, Sharifi et al. developed PCL microfibers using a microfluidic approach, obtaining tunability in mechanical properties and porosity, paving the way for their use in tissue engineering applications [103]. Mass production of highly uniform microparticles using a parallelized microfluidic device was achieved in 2018 by Yadavali et al., reaching production times one thousand times faster than existing parallelized devices [104] (See Fig. 11A). In 2024, core-shell drug loaded nanocapsules were obtained using a self-assembly method that involved the co-precipitation of the target drug, oil, PCL and PCL-PEG [68].

3.2. Hydrogels

Among different material classes, hydrogels have been used for various applications, such as drug delivery, wearable sensors, 3D cell culture matrices, etc. thanks to their properties [105,106]. They are hydrophilic, three-dimensional and crosslinked polymer systems that show high swelling capabilities that can be tuned depending on the use.

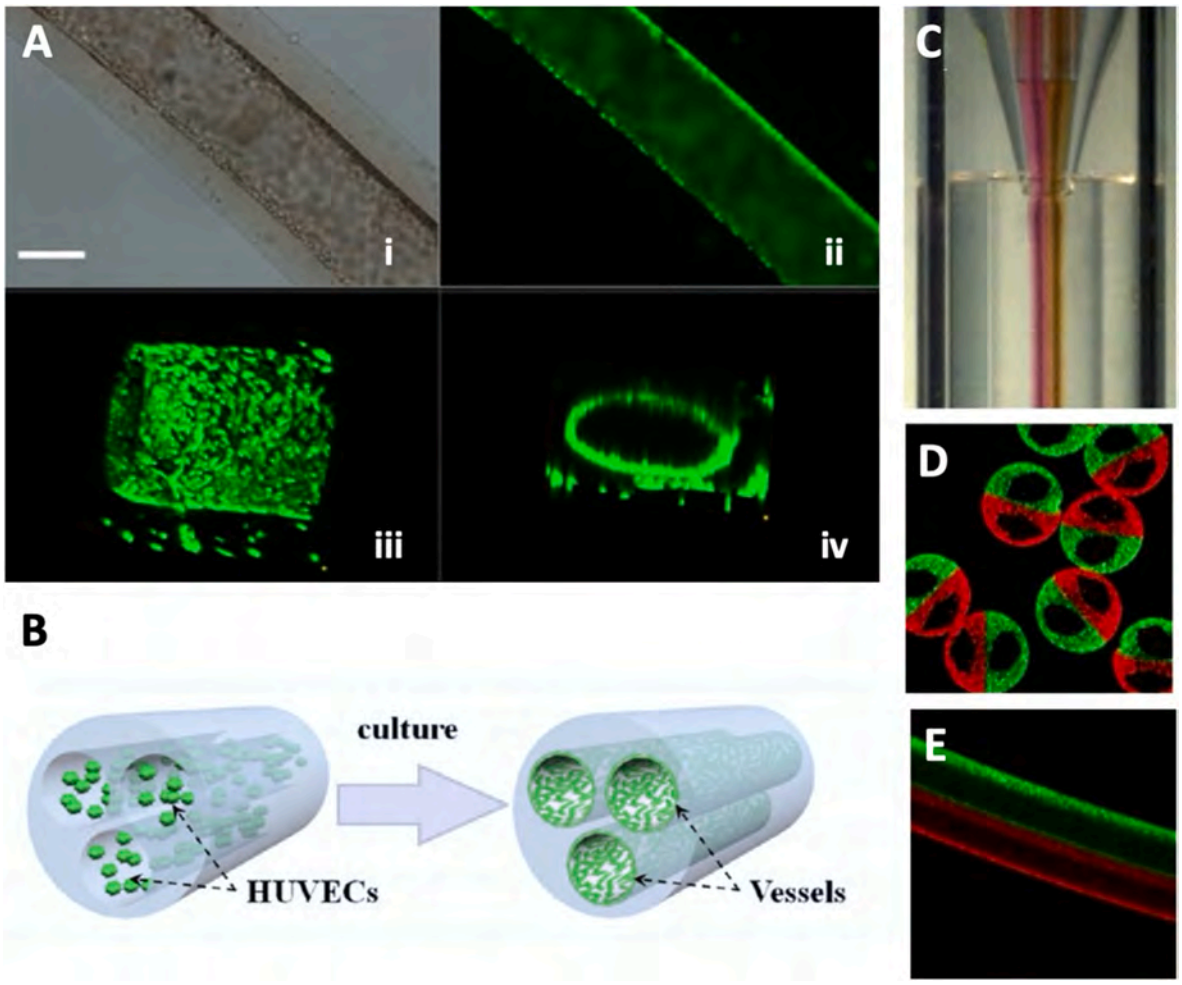

Fig. 9. Fabrication of bioactive microfibers using a multibarrel microfluidic device. (A) GelMA hollow microfiber seeded with HUVECs: (i) optical image, (ii) fluorescent image, and (iii–iv) Confocal Laser Scanning Microscopy (CLSM) reconstructions from different viewpoints (Scale bar: 200 μm). (B) Schematic of biomimetic vessel formation after cell culture within the hollow lumen. (C) Photograph of the microfluidic device used to fabricate Janus and hollow fibers by modulating inlet configurations. (D–E) Confocal images showing longitudinal (D) and cross-sectional (E) views of microfibers with spatially patterned hydrogel compartments [86].

Table 2
Comparative summary of key biomaterial classes for DBM.

Biomaterial type	Advantages	Limitations
Solid polymers	Good mechanical stability; controlled degradation; high reproducibility	Limited biocompatibility without surface modification; hydrophobic nature
Natural hydrogels	Excellent biocompatibility; bioactivity; mild gelation	Poor mechanical strength; batch-to-batch variability
Semi-synthetic hydrogels	Tunable mechanics; photopatternable; cell-adhesive motifs	Requires photoinitiators (possible cytotoxicity); requires external systems for crosslinking
Synthetic hydrogels	Highly reproducible; chemically versatile; controlled properties	Lacks inherent bioactivity; needs functionalization or combination with natural hydrogels

Hydrogels form semi-solid or solid gel structures, mantaining mechanical integrity. Moreover hydrogels mimick the extracellular matrix (ECM) and retain fluids, like cell culture media. Those characteristics are essential to enable nutrient exchange in 3D cell culture [90]. Hydrogels can be classified as natural, semi-synthetic, or synthetic. Gelation techniques play a crucial role in determining the final properties of hydrogels, as they directly impact the mechanical strength, structure, and functionality of the material. The choice of gelation method influences various factors, including the stability, uniformity, and bioactivity of the hydrogel. Depending on the specific technique employed, different requirements must be considered for the microfluidic devices used in hydrogel formation. The main gelation techniques are outlined below.

3.2.1. Gelation techniques

3.2.1.1. Chemical crosslinking. Chemical crosslinking is a process in which the molecules of a biomaterial, are crosslinked through ionic interactions. These interactions occur between the hydrogel and metal ions and promote the formation of bonds between the polymer chains, stabilizing the material's structure and inducing the formation of a gel. A common example of ionic gelation is the crosslinking of alginate with Ca^{2+}, which is widely used in droplet-based microfluidic applications for producing monodisperse microbeads [107]. In DBM devices, ionic gelation is implemented by introducing a polymer solution (such as alginate or chitosan) into a flow containing calcium ions or other crosslinking ions [108]. The shape of the microfluidic device and the flow conditions allow for the generation of uniform droplets that gel rapidly upon contact with the ions, forming microbeads. Naturally, the uniformity of the microspheres in terms of circularity and monodispersity will vary depending on the amount and concentration of the

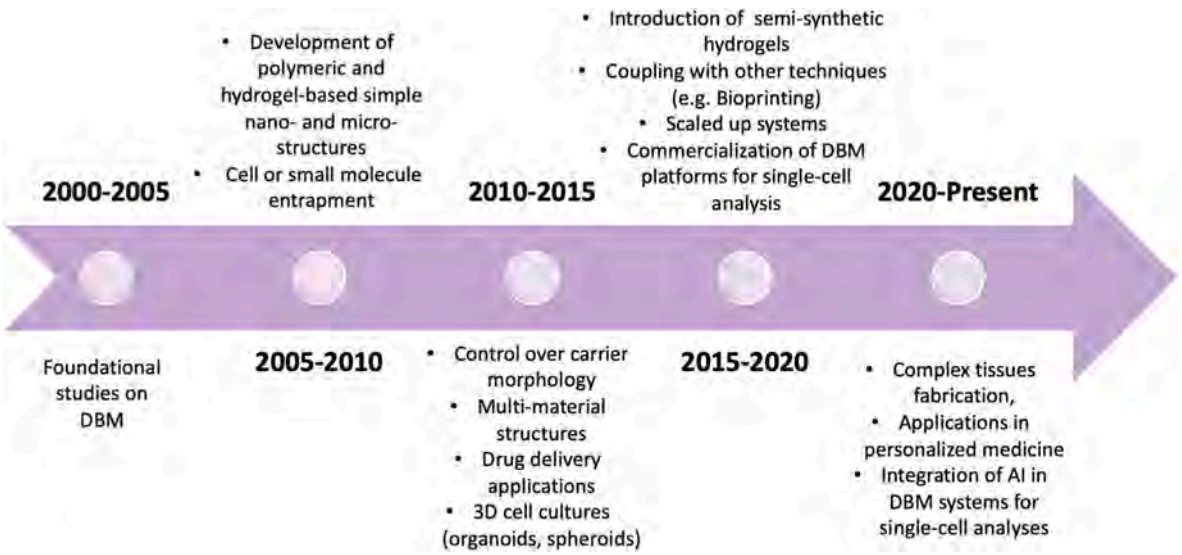

Fig. 10. Key milestones in the development of DBM for biomaterial production, from early proof-of-concept studies to recent advances in biomedical applications.

Table 3
Applications of solid polymers in DBM.

Material	Structure	Aim/Results	Reference
PLGA	Microfibers	Cell orientation studies	[96]
	Nanoparticles	Better control over traditional drug-	[64]
	Nanoparticles	delivery systems Drug-delivery	[97]
	Nanoparticles	Introduction of ultrasonic emulsion in DBM	[98]
PCL	Microcapsules	Encapsulation of drugs and	[101]
	Microparticles	nanoparticles	[102]
	Microfibers	Combination of experimental and computational data Morphology tunability for tissue engineering applications	[103]
	Microparticles	Mass production using parallelized devices	[104]
	Core Shell nanocapsules	Stealth nanocapsules for drug delivery	[68]

crosslinking agent, whether internal or external ionic gelation is applied [109,110]. This process is advantageous because it allows for rapid gelation at room temperature, avoiding the need for extreme thermal conditions or complex chemical reagents. Additionally, ionic gelation is easily controllable by adjusting the ion concentration, the geometry of the microfluidic channels, and the flow rate. However, a limitation of this technique is that produced materials, such as alginate, are often not bioactive, requiring chemical modifications to improve cell adhesion and biological functionality. Moreover, depending on the cell line used in case of encapsulation, the presence of ions during the production of those systems may alter their physiological response, posing a further limitation of this crosslinking method. For instance, hepatocellular carcinoma (HCC) cells encapsulated in alginate beads using a 200 mM Ca^{2+} crosslinking solution showed improved invading ability and increased expression of metastasis-related genes compared to beads formed with 50 mM Ca^{2+} [111].

3.2.1.2. Enzymatic gelation. Enzymatic gelation is a process in which covalent bonds are formed between the molecules of a biomaterial through catalysis by specific enzymes, inducing gelation. Unlike traditional chemical gelation techniques, which require external chemical agents, enzymatic gelation exploits the biological activity of enzymes to promote the crosslinking of polymer chains [112]. In droplet-based microfluidic devices, enzymatic gelation is implemented by introducing a specific enzyme into the biomaterial flow [113]. When the polymer (such as hyaluronic acid or gelatin) comes into contact with the enzyme, gelation occurs, which can be highly controlled based on enzyme concentration and environmental conditions, such as pH or temperature. This technique is particularly advantageous for biological applications, as it can produce hydrogels that are highly biocompatible and biodegradable, ideal for cell encapsulation and tissue manipulation. However, enzymatic gelation may be limited by the availability of specific enzymes and the difficulty of controlling the reaction in real-time, especially when a long-lasting gel is required. Despite these challenges, enzymatic gelation offers a very promising solution for the creation of functional biomaterials in microfluidic systems.

3.2.1.3. Physical gelation. Physical gelation is based on non-covalent interactions, such as hydrogen bonds, electrostatic forces, and hydrophobic interactions, which promote the formation of gelable structures without the need for direct chemical reactions [114]. In this technique, biomaterials such as agarose, gelatin, and silk fibroin form gels through physical processes like cooling, self-assembly, or modification of environmental conditions (pH, temperature, ionic forces). Thermal gelation, for example, is a common process in which a biomaterial such as agarose forms a gel at low temperatures, becoming liquid when heated and solid at ambient temperature or lower [115]. In droplet-based microfluidic devices, physical gelation is implemented by controlling environmental conditions, such as temperature or pH, within the microchannels. In the case of agarose, which is a thermoresponsive polymer, it is widely used in the DBM context to create reversible microparticles that can be dissolved at physiological temperature after formation. This process allows the generation of microbeads that can be easily manipulated and offer good mechanical stability. However, physical gelation has limitations in terms of reproducibility of mechanical properties and gel stability, as it is highly dependent on surrounding environmental conditions. Furthermore, some biomaterials, such as agarose, lack bioactivity and must be modified for biological applications, such as cell encapsulation.

3.2.1.4. Photocrosslinking. Photocrosslinking gelation is a process that uses UV radiation to trigger the formation of covalent bonds between the molecules of a biomaterial, inducing polymer crosslinking and gel formation [116]. In this technique, biomaterials are chemically modified to include photoactivatable functional groups, such as acrylic or methacrylic groups, that react with UV light to create crosslinks between polymer chains. A common example is the use of GelMA, a derivative of gelatin that combines thermal gelation and photocrosslinking, allowing precise control over gel formation and its mechanical properties [85]. In

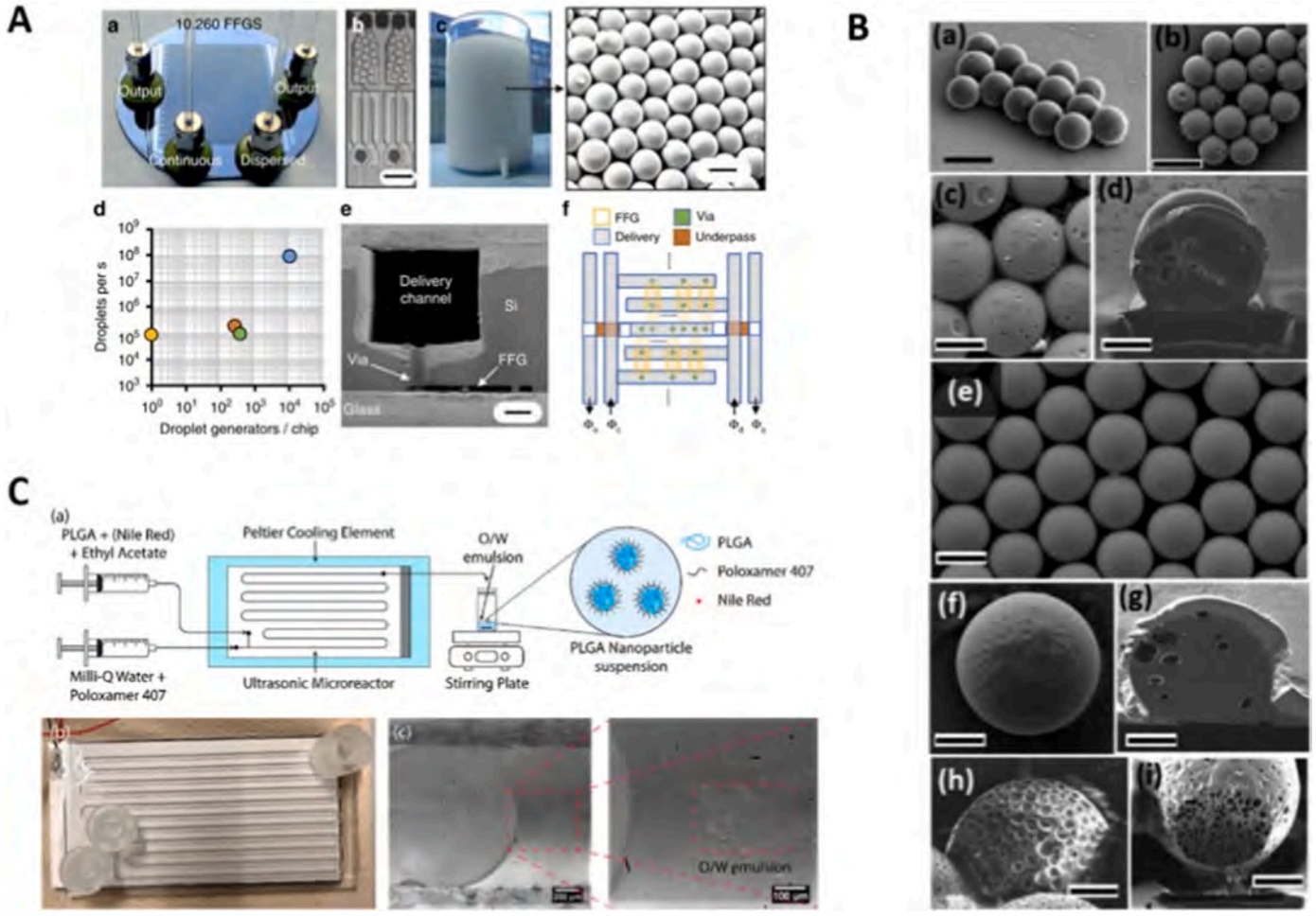

Fig. 11. Examples of solid polymer applications in DBM. (A) Very large scale droplet integration (VLSDI) device integrating 10260 parallelized flow-focusing PCL droplet generators (Scale bars: 140 μm (a), 8 μm (d) and 90 μm (e)) [104]; SEM images of dimped PLA particles under different conditions at different PLGA concentrations (Scale bars: (a and b) 20, (c) 9, (d) 6, (e) 20, (f) 4.5, (g and h) 44, and (i) 50 μm) [75]; (C) Schematics of PLGA microparticles synthesis with the aid of an ultrasonic microreactor (Scale bars: 200 μm, 100 μm (c).) [98].

droplet-based microfluidic devices, photocrosslinking gelation is implemented by introducing a solution containing the photoactivatable biomaterial into microchannels, where it is irradiated with UV light to activate crosslinking. This approach offers very precise control over the gel's size and structure, which can be adjusted according to the application's requirements. Photocrosslinking is particularly advantageous in applications that require rapid gel formation, such as cell encapsulation, as it allows for very fast and highly controlled gel production. However, a potential drawback of this technique is that UV radiation can be harmful to cells or other sensitive biological components, necessitating careful optimization of irradiation conditions to avoid damage. Additionally, the solubility of biomaterials may be affected by UV treatment, particularly for materials like PEGDA, which are commonly used for their excellent crosslinking properties in microfluidics [117].

3.3. Natural hydrogels

Natural hydrogels offer high biocompatibility; being water-based materials they allow live cell encapsulation, unlike solid polymers [118]. Except agarose and alginate, natural hydrogels contain adhesion motifs to enhance cell adhesion, proliferation and differentiation [90]. Their downside is their poor mechanical properties and batch-to-batch variability [119]. The most used natural materials are polysaccharides (agarose, alginate, chitosan, hyaluronic acid, xantham gum) or proteins (collagen, gelatin). Table 4 resumes various studies involving natural hydrogels in DBM.

3.3.1. Agarose

Agarose is a linear polysaccharide, derived from red seaweed. In solution, agarose forms a thermoresponsive hydrogel, undergoing physical gelation at temperatures below 35 °C [120]. It can be used to produce reversible microparticles that can be dissolved at physiological temperature. The enhanced tunability of this simple material however is counteracted by its lack of bioactivity and its non-biodegradability by mammalian enzymes [121]. Huebner et al. (2007) used agarose for the first time in microfluidic devices, compartmentalizing single-cells, cell-cell or cell-subpopulations in aqueous droplets [122]. Kumacheva et al. (2011) developed a device that generates a library of hydrogel microenvironments with tunable elasticity for cell encapsulation [123]. The same group (Kumacheva et al. 2014) later investigated the combined use of agarose and chitosan, obtaining dual pH and temperature responsive microparticles for drug delivery [124]. Agarose microparticles have also been used by Eun et al. (2011) for the encapsulation of bacteria such as Escherichia Coli (E. Coli) with a range of application from highthroughput screening for target proteins to human microbiome studies and phenotypebased screening of bacteria [125]. A more complex device by Shi et al. (2013) generated and further trapped the microparticles, allowing the production of an array of microparticles with different components [126]. More recently, in 2025, George et al. used agarose in combination with gellan gum inside a pump-free

Table 4
Applications of natural hydrogels in DBM.

Material	Structure	Aim/Results	Reference
Agarose	Microbeads	Single cell or cell subpopulation encapsulation	[122]
	Microspheres	Tuneable elasticity simulating different microenvironments	[123]
	Microparticles	Drug delivery	[124]
	Microparticles	Bacteria encapsulation and human microbiome studies	[125]
	Microparticles	Array with different components for cell culture	[126]
	Microparticles	DNA-amplification for high-throughput diagnostics	[127]
Alginate	Microbeads	Monodispersed structures	[131]
	Microspheres	RGD-modification of alginate supported single-cell encapsulation	[132]
	Microparticles	High-throughput encapsulation of stem cell spheroids further seeded on PLA scaffolds	[133]
	Microparticles	BSA and TGF-β3 encapsulation and *in vitro* release	[134]
Chitosan	Microparticles	Ampecillin encapsulation	[139]
	Microfibers	Scaffold for tissue culture	[140]
	Microparticles	MSCs culture	[141]
	Microparticles	hMSCs culture and stimulation	[142]
	Nanoparticles	β-Galactosidase, mRNA and siRNA encapsulation	[143]

Material	Structure	Application/Results	Reference
Collagen	Microparticles	MDA-MB231 encapsulation mESCs and ovarian preantral culture	[146]
	Core-shell	Breast Spheroid culture	[147]
	Microparticles		[148]
	Core-Shell		
	Microparticles		
	Microspheres	Scaffold for endothelial culture	[149]
	Microbeads	Tissue-engineered bone grafts incorporating vascular compartment	[150]
	Microtissue Spheroids	Lobule-like structure maturation	[151]
Gelatin	Microcapsules	Cell encapsulation	[154,155]
	Core-shell	Controlled stiffness of shell and core	
	Microparticles		
	Microspheres and microrods	Structure tunability, oil-free production method	[156]
	Microspheres	Enhanced drug release in bone cements	[157]
Hyaluronic Acid	Microfibers	Drug delivery	[159]
	Microspheres	Cell encapsulation	[160]
	Microparticles	Optimization of HA crosslinking and process tuneability with click-chemistry	[161]

Material	Structure	Application/Results	Reference
Matrigel	Microspheres	Acina-like structures maturation	[165]
	Microbeads	Spheroids and organoids encapsulation	[166,167]
	Core-shell	Enhanced viability respect to solid alginate carriers	
	Microparticles		
	Microbeads	Multicompartmental cancer invasion model	[168]
	Assembloids	Maturation of patient-derived assembloids for high-throughput screening applications	[169]
Silk Fibroin	Microspheres	Monodispersed structures	[171]
	Micropshere	Drug encapsulation	[172]
	Nanoparticles	Characterization and macrophage response assessment Drug delivery	[173]
	Microparticles		[174]
	Janus-like	Structure characterization	[175]
	Microparticles		
	Microspheres	Bone regeneration	[176]

microfluidic device that served as microreactor for on-chip nucleic acid amplification enabling high-throughput, low-volume diagnostics [127].

3.3.2. *Alginate*

Alginate is an anionic polysaccharide obtained from brown seaweeds. The main strength of alginate is the immediate cross-linking when exposed to calcium ions (Ca^{2+}), resulting in a low-stress cross-linking mechanism for embedded cells [128]. Ionic concentration together with flow and geometry control allows the fine tuning of alginate microcarriers [129]. However, alginate is not bioactive as it does not contain any cell adhesive moiety [130]. Despite not being bioactive, alginate has been widely employed in the generation of bioactive systems, being chemically modified or in combination with other materials. The application of alginate-based droplets ranges from single-cell encapsulation to bioactive molecules delivery systems [130]. The first study on sufficiently monodispersed alginate-based microbeads was reported by Tan et al. (2007), but lacked cell adhesivity and the possibility to automatically handle the microbeads [131] (See Fig. 12A). Shao et al. (2020), developed a microfluidic device for single-cell encapsulation in arginin-glycine-aspartic acid (RGD)-modified alginate, overcoming the bioinertness of unmodified alginate [132]. Mequsita et al. (2021) used alginate-based homogeneous microparticles for the encapsulation of stem cell spheroids, reaching a throughput of 200 droplets/min, further seeding the obtained microparticles on PLA scaffolds [133] (See Fig. 12C). Alginate-based microparticles have also been used by Trinh et al. (2021) as Bovine Serum Albumin (BSA) and Transforming Growth Factor β3 (TGF-β3) carriers to induce *in vitro* chondrogenesis of mesenchymal stem cells [134].

3.3.3. *Chitosan*

Chitosan is a cationic polysaccharide, derived from chitin. Its gelation is mediated by polyphosphates and aldehydes [135]. Chitosan has good biocompatibility, antibacterial and bioadhesive properties [136]. Also, chitosan is biodegraded by lysozyme and other mammalian enzyme, making it a good candidate for temporary scaffolds [137]. However, chitosan showed poor mechanical properties and it is often used in combination with other materials or chemically modified [138]. Yang et al. (2007) firstly introduced monodisperse chitosan microparticles produced with the aid of a microfluidic chip, namely for encapsulation of ampecillin [139]. Early reports of chitosan used in microfluidic systems also involved the production of microfibers used as scaffold for cell culture [140] (Yeh et al.(2009)). Shin et al. (2023) developed a device for microchannel emulsification, encapsulation of mesenchymal stromal cells in unmodified chitosan, maintaining high

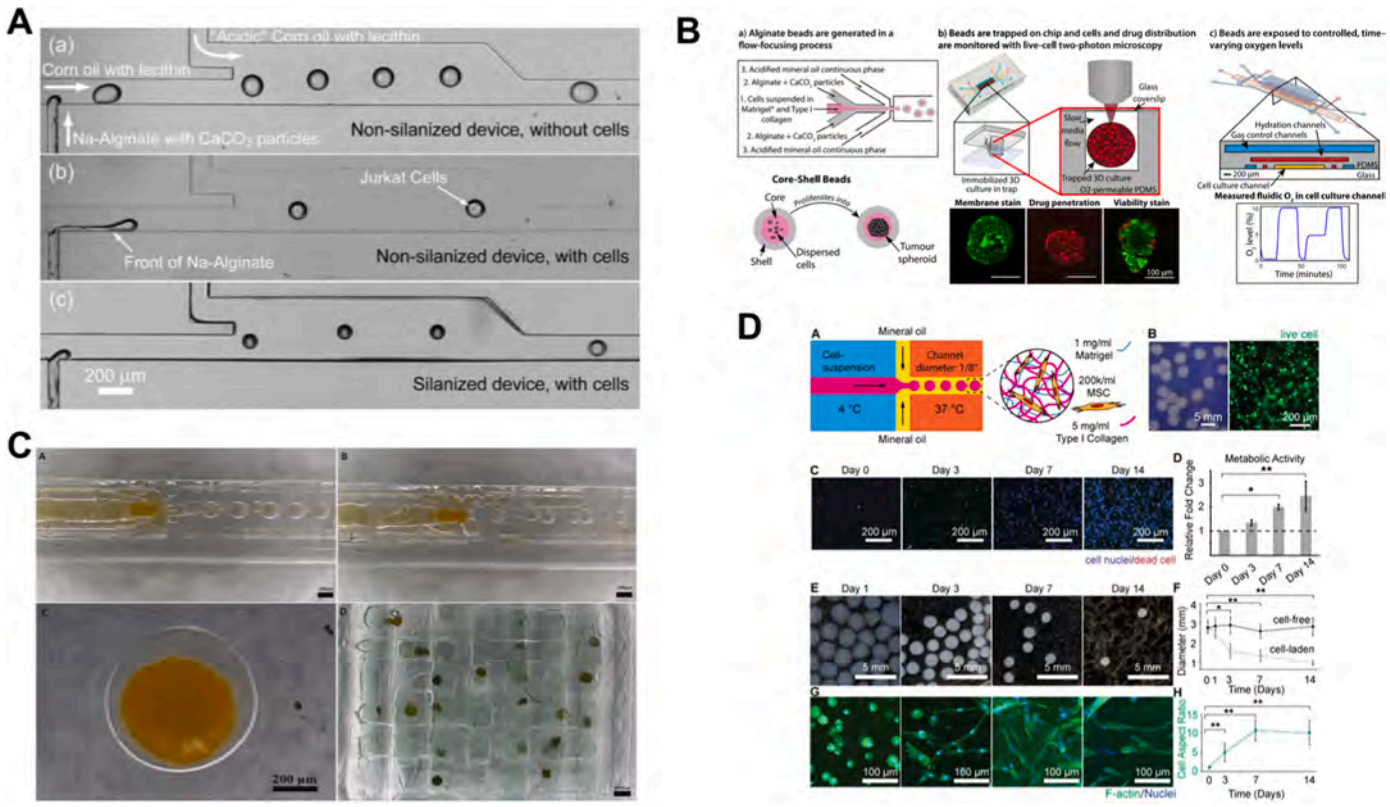

Fig. 12. Examples of natural hydrogels applications in DBM. (A) Optical microscopy images of alginate droplets formation in a microfluidic device under different conditions [131]; (B) Microfluidic system for tumour spheroid generation, culture, oxygen control and microscopy monitoring using core-shell alginate beads (Scale bar: 100 µm) [148]; (C) Encapsulation of stem cell spheroids in alginate microspheres and further embedding in PLA scaffolds (Scale bars: 200 µm (A), (B), (C), 2000 µm (D).) [133]; (D) Schematic of collagen/Matrigel microbeads preparation and characterization of MSCs-laden carriers [150].

cell viability and functionality even after passing through 21G needles, demonstrating better control and stability with respect to traditional stirred-emulsion fabricated chitosan microparticles [141]. San Roman et al. (2021) encapsulated human mesenchymal stem cells (hMSCs) using antioxidant glycerylphtate (G_1Phy) and triplolyphosphate (TPP) as crosslinker agents. Moreover, G_1Phy acted as upregulator of paracrine factor secretion, introducing a bioactive function of the microcarriers [142]. Greco et al. (2023) developed a microfluidic mixer for the production of chitosan nanoparticles, loading them with different cargos such as β-Galactosidase, messenger Ribonucleic Acid (mRNA) and small interference Ribonucleic Acid (siRNA) [143].

3.3.4. Collagen

Collagen is the most abundant component in connective tissue [144]. It is a structural protein fundamental in ECM composition, showing excellent biocompatibility, low antigenicity and great bioadhesive properties, showing also biodegradability [144]. Although it can reach higher mechanical stability compared to other natural hydrogels, its crosslinking is pH-sensitive and slow and it leads to contraction of the obtained structures [145]. For this reason, collagen is often used in combination with other materials. The first use of collagen in microfluidic devices dates back to a study by Hong et al. (2012). The authors developed a chip for the droplet generation, gelation and extraction for the encapsulation of breast cancer cells MDA-MB231 obtaining good size distribution and high cell viability after encapsulation [146]. Agarwal et al. (2015) developed a microfluidic device for the generation of core-shell microparticles for the encapsulation of mouse embryonic stem cells (mESCs) and ovarian preantral follicles. The devices exploited alginate as a containing shell for the biomimetic core, type I collagen, that was tested with cells that shows vastly different ECM compositions in vivo, demonstrating the capability of collagen to support viability and proliferation of different cell lines in the same microcarrier [147]. Similarly, collagen was used as biomimetic core in another study by Grist et al. (2019) for the maturation and long-term culture of breast tumor spheroids [148] (See Fig. 12B). Another approach from Crampton et al. (2018) involved collagen in the production of microspheres successively coated with endothelial cells to obtain a microtissue platform for *in vitro* studies [149]. More recently, collagen microstructures have been used synergistically together with bioprinting. Yang et al. (2021) used collagen-based beads to obtain tissue engineered bone grafts (TEBGs), integrating a vascular compartment with the aid of extrusion-based bioprinting (EBB) [150] (See Fig. 12D). Hong et al. (2021) combined DBM and EBB to obtain hepatic spheroids that exhibited a biomimetic lobule-like structure [151].

3.3.5. Gelatin

Gelatin is a protein derived from collagen available from an extensive range of sources (porcine, bovine, fish) [118]. Gelatin is a thermoresponsive hydrogel, that forms solid-like gels at low temperatures and forms a sol-like gel at body temperature. Unlike collagen, gelatin is highly soluble at physiological pH [152]. Gelatin can be used on its own or as additive to provide adhesive properties thanks to its naturally occurring RGD peptide sequence, notably known as adhesive site for cells [153]. Gelatin was firstly employed to produce microcapsules for different cell lines by Sakai et al. (2011); to stabilize gelatin microcapsules, a modified enzymatically crosslinkable gelatin was used as a coating after production and physical gelation at 4 °C [154]. Knowles et al. (2020) obtained gelatin-based core-dense and shell-dense microgels for different applications, from tissue engineering to drug delivery, reaching controlled radial density, thermal stability and differential degradability [155]. In a further work the same group [156] successfully developed a liquid-liquid phase-separated model eliminating the need

for oil in traditionally used oil/water emulsion systems, obtaining both drop-like structures and microrods. Wang et al. (2024) utilized DBM to synthesize chemically crosslinked gelatin microcarriers for the enhancement of drug release in bone cements [157].

3.3.6. Hyaluronic acid (HA)

HA is a naturally occurring component of the ECM. It has optimal swelling properties and biocompatibility [158]. HA is often used in systems that generate droplets for cellular migration studies, tissue engineering and regenerative medicine. HA was also used by Agnello et al. (2016) to obtain microfibers for drug delivery exploiting enzymatic crosslinking, dexamethasone was used as encapsulated drug [159]. Ma et al. (2017) used enzymatic crosslinking and Diels-Alder click chemistry to obtain HA-based cell carriers. The resulting carriers showed tailored properties suitable for cells encapsulation [160]. Different chemical modification were investigated to make HA available for covalent crosslinking by Heida et al. (2020), optimizing the production of tailored carrier-structures for further applications [161]. Del Giudice et al. (2023) extensively studied the viscoelastic encapsulation of particles and cells using HA not as a carrier but as a suspending liquid for the particles formation [162].

3.3.7. Matrigel

Matrigel is a basement membrane extract derived from mouse sarcoma cellsm, and is widely used to emulate the extracellular matrix composition, as it contains laminin, collagen IV and different proteoglycans [163]. Matrigel rapidly gels at 37 °C as its constituent proteins self-assemble, leading to difficulty in handling this particular material [164]. First used by Dolega et al. (2015), a flow focusing device placed in a cold room (4 °C) was able to generate carrier for epithelial cells forming acina-like structures [165]. To avoid low-temperature stresses for the embedded cells, Laperrousaz et al. (2018) used a smaller benchtop cooling device and successfully generated spheroid or organoid laden Matrigel microbeads [166]. Li et al. (2018) demonstrated the enhanced cell response comparing simple alginate microparticles and core-shell microparticles with an alginate shell and cell-embedding Matrigel core, obtaining significant difference in viability [167]. Jouybar et al. (2023) integrated a temperature-controlled microfluidic flow-focusing device that allowed in situ gelation of the obtained microbeads. Matrigel microbeads containing cancer cells were then embedded with a sandwiching approach in collagen sheets that simulated stroma, obtaining a faithful model of cancer invasion [168]. More recently, Zhang et al. (2024) used a combination of Metrigel and GelMA to obtain patient-derived lung cancer assembloids for applications in high-throughput therapeutic screenings [169].

3.3.8. Silk fibroin (SF)

SF is a protein derived from silkworm *(Bombyx Mori)* cocoons. Despite being a natural hydrogel, its highly organized structure results in enhanced mechanical properties [170]. The first reported use of SF for the production of microcarriers, monodispersed and tunable in size is by Braslauer et al. (2010) [171]. Drug-encapsulation and release ability of silk fibroin microspheres was demonstrated by Mitropoulos et al. (2014), using albumin-fluorescein isothiocyanate conjugate (FITC-BSA) and fluorescein isothiocyanate conjugated dextran as loads [172]. Wongpiyochit et al. (2018) developed the first device that allowed a continuous manufacture of silk nanoparticles, testing their uptake by macrophages [173]. Montoya et al. (2020) tested DOX-loaded silk fibroin particles effect on a neuroblastoma cell line (SK-N-AS), demonstrating citotoxicity; also, macrophages uptake of silk particles was reported, inducing a pro-inflammatory state [174]. Janus-like microparticles were obtained using silk fibroin in a study by Toprakcioglu et al. (2021), obtaining an hard shell and a liquid core by exploiting shear-mediated sol-gel transition [175]. Luetchford et al. (2020) used a gelatin/silk fibroin combination to obtain microcarriers for bone regeneration application, demonstrating good cell adhesion and proliferation [176].

3.4. Semi-synthetic hydrogels

Chemical modification of natural materials generated a sub-class of materials used in the biomedical field, often referred to as semi-synthetic materials [118]. Semi-synthetic hydrogels maintain the advantages of natural materials enhancing their mechanical properties [177]. Among the most common modifications, methacrylation allows to obtain photo-curable materials that can be easily and rapidly crosslinked [178]. All the various studies involving semi-synthetic hydrogels in DBM are summarized in Table 5.

3.4.1. Gelatin methacryloyl (GelMA)

GelMA is a gelatin derivative that has been extensively used in the biomedical field for a wide range of applications [179]. GelMA combines the physical gelation mechanism and UV-cross-linking to allow fine-tuning of its processability [118]. GelMA was used by Cheng et al. (2016) as a bioactive additive for the production of multi-compartmental alginate fibers for endothelial cells (HUVECs) culture [86]. Parker et al. (2016) first used GelMA to obtain monodispersed droplets using a relatively simple flow focusing device [180]. A more complex device developed by Mohamed et al. (2019) integrated cell-laden droplet formation, gelation, removal of the residual oil in a single device [181]. Encapsuled cells then demonstrated growth, proliferation, cell-cell interaction and high viability. Wang et al. (2019) used a flow-focusing device to fabricate GelMA microcapsules encapsulating cancerous hepatic cells (HepG2), demonstrating the ability to support proliferation and albumin and urea secretion [85] (See Fig. 13C). Chen et al. (2023) extensively studied GelMA microcarriers for stem cell culture from encapsulation to differentiation and stemness maintenance of bone marrow stem cells (BMSCs), outperforming commercially available carriers [182] (See Fig. 13A).

3.4.2. Hyaluronic acid methacryloyl (HAMA)

Costa-Almeida et al. (2017) fabricated multi-component HAMA-based fibers for the development of tissues that display fibrillar-like structures such as tendons [183] (See Fig. 13B). HAMA microgels were introduced by Busatto et *et al.* (2017) and has been one of the first examples of oil-droplets carrying microgels for the release of hydrophobic drugs [184]. Most recently, Roh et al. (2024) successfully encapsulated single extracellular vesicles (EVs) for protein profiling, overcoming the many limitations of currently available platforms for single EV analysis methods [185] (See Fig. 13D).

41.

3.5. Synthetic hydrogels

Synthetic hydrogels overcome the limitations of natural hydrogels. Those hydrogels are synthesized by chemical reaction, guaranteeing

Table 5
Applications of semi-synthetic hydrogels in DBM.

Material	Structure	Application/Results	Reference
Gelatin methacryloyl	Microfiber	Multi-compartimental fiber	[86]
	Microsphere	for endothelial cell culture	[180]
	Microsphere	Monodispersed microspheres	[181]
		Integration of all fabrication processes in one device	
	Microcapsule	Hepatic cell culture	[85]
	Microsphere	Stem cell culture	[182]
Hyaluronic Acid methacryloyl	Microfiber	Cell culture for tissue	[183]
	Microgel	engineering	[184]
		Drug Delivery	
	Microsphere	Extracellular Vesicle Analysis	[185]

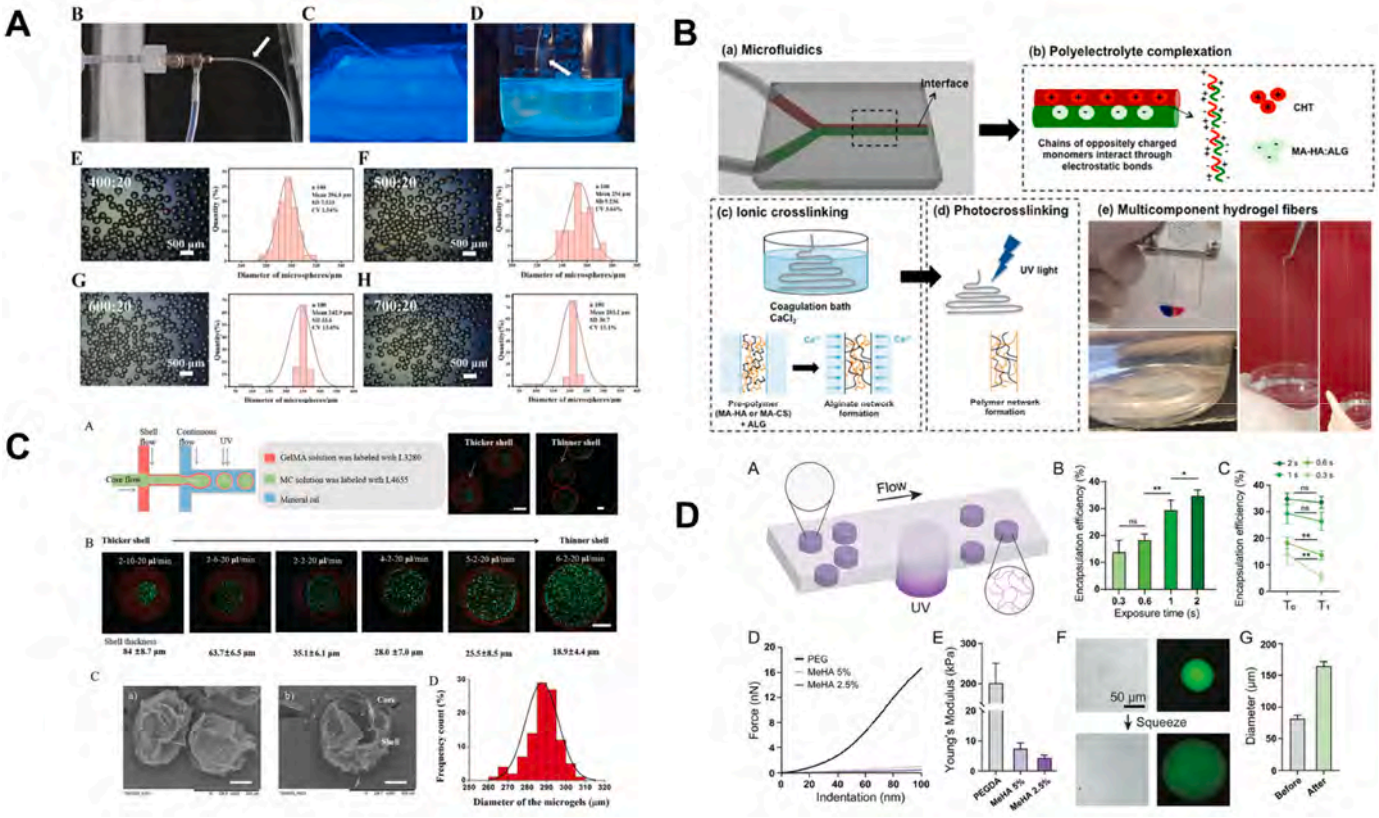

Fig. 13. Examples of semi-synthetic hydrogels applications in DBM. (A) Schematics of synthesis and morphology characterization of GelMA microcarriers. The microfluidic setup includes synchronous photo-crosslinking of GelMA during fabrication [182]; (B) Schematics of the fabrication process of multicomponent hydrogel fibers. [183]; (C) Morphological characterization of GelMA core-shell microspheres for cells encapsulation (Scale bar: 100 μm (A), (B), (C).) [85]; (D) Preparation schematics and characterization of squeezable HAMA microspheres [185].

Table 6
Applications of synthetic hydrogels in DBM.

Material	Structure	Aim/Results	Reference
PEG	Microdroplets	Bacterial Encapsulation	[188,
	Microspheres	Protein and DNA nanoparticles delivery	189]
	Microdroplets	Water-in-water droplet generation	[190]
	Microdroplets	Magnetic droplets manipulation	[191]
	Microcapsules	Cell encapsulation for cell-based therapies	[192]
PVA	Microspheres	Cell encapsulation	[196]
	Microspheres	Developing a freeze-thaw method to	[197]
	Microspheres	produce drug carriers	[198]
	Microspheres	Metallic nanoparticles entrapment Drug Delivery	[199]
	Microspheres	Drug carrier for embolization therapies	[200]
	Microspheres	Comparison with PLGA and alginate carriers	[201]
PAAm	Core-Shell	Size and morphology high tunability	[204]
	Microcapsules	Switchable elasticity for mechano-	[205,
	Core-Shell	responsive scaffolds	206]
	Microcapsules Microspheres	Monodispersed PAAm-only droplets	
	Microspheres	DNA-Barcoding	[207]
	Microspheres	Mass production using parallelized devices and droplet splitting	[208]

optimal batch-to-batch consistency, avoiding risks of toxicity or immunogenicity, and providing better mechanical properties [186]. However, synthetic hydrogels often lack the adhesive properties and biocompatibility of natural hydrogels [186]. All the various studies involving semi-synthetic hydrogles in DBM are summarized in Table 6.

3.5.1. *Polyethylene glycol (PEG) and derivatives*

PEG is an FDA-approved biomaterial that is hydrophilic, biocompatible, non-immunogenic, and with highly tuneable mechanical or chemical properties. PEG properties can be tuned modifying its structure (linear, branched, multi-arm) or by chemical modifications, introducing different functional groups such as dehydrogenase (PEGDHS), methacryloyl (PEGMA), diacrylate (PEGDA), etc. PEG can be also used to functionalize surfaces or micro/nanoparticles, to create stealth surfaces [187] thanks to its protein adsorption and cell adhesion resistance. In particular, PEGDA is widely used in droplet-generating microfluidic devices thanks to the easily tunable properties and fast crosslinking when exposed to ultraviolet (UV)-light. In 2010, Lee et al. encapsulated E. Coli in PEGDA microparticles, maintaining high viability and fluorescent protein expression [188]. For controlled drug-delivery applications, in 2015 Deveza et al. used PEG to encapsulate and release grow factors and DNA nanoparticles while maintaining their bioactivity with tunable release kinetics [189]. Water-in-water production of PEG droplets was achieved by Jeyhani et al. in 2019, overcoming water-in-oil droplet systems negative effects on biological cargoes such as cells [190]. Using a water-in-water system, Navi et al. in 2020 achieved magnetic manipulation of droplets for enhanced sorting and guidance capabilities [191]. Furthermore, PEG-tyramine microcapsules have demonstrated significant clinical potential for diabetes therapy, encapsulating β-cells, providing immunoprotection and maintaining glucose responsiveness for extended periods [192] (See Fig. 14D).

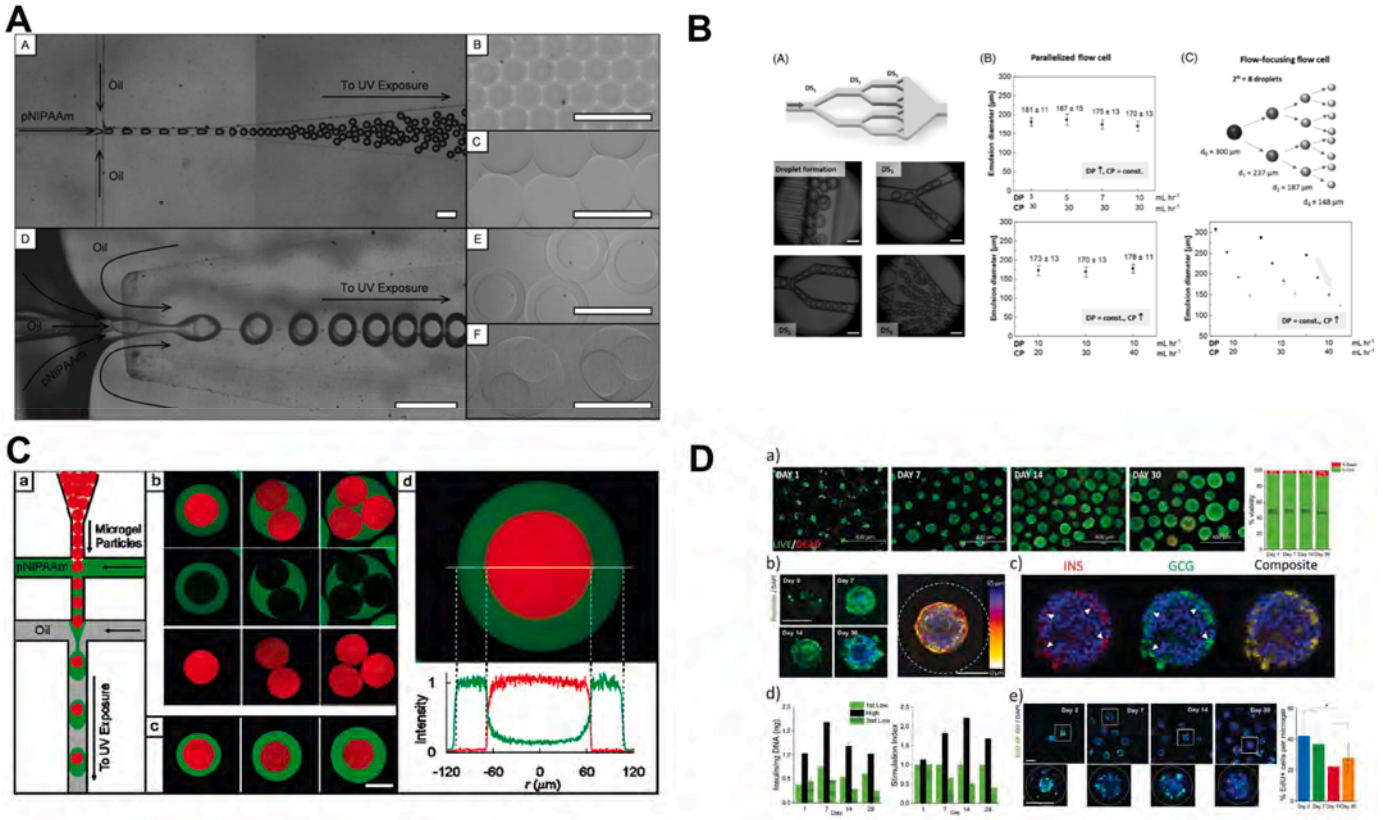

Fig. 14. Examples of synthetic hydrogels applications in DBM. (A) Monodisperse particles generated from semidilute pNIPAAm precursor solutions followed by UV-induced polymerization. Variations in channel geometry and flow rates enable control over particle size and structure (All scale bars: 200 μm) [202]; (B) CAD model of the sequential droplet splitter (DS) with bright-field images showing droplet generation and splitting (Scale bars: 300 μm) [208]; (C) Schematics of core-shell pNIPAAm droplets loaded with pNIPAAm or polyacrylamide particles. Fluorescence intensity profiles confirm minimal interpenetration between core and shell layers (Scale bar: 100 μm). [204]; (D) Characterization of β-cells inside hollow PEG-TA microgels (Scale bars: 400 μm (a), 100 μm (b,e).) [192].

3.5.2. Polyvinyl alcohol (PVA)

PVA is a synthetic hydrogel that can be either chemically or physically crosslinked. PVA is widely used for drug delivery. wound healing, bioprinting, tissue scaffolding etc. [193]. When not crosslinked, PVA can be used as additive in emulsion or as a support material thanks to its high water solubility. The first uses of PVA in DBM were mainly involving PDMS channel coatings to induce hydrophilic surfaces [194,195]. In 2013, Young et al. developed PVA and a combination of PVA and heparin-based microcarriers for L929 murine fibroblast [196]. Xian-Wei *et al.*developed in 2018 a method to obtain physically crosslinked PVA microspheres by freezing and thawing them; good monodispersity and size-tunability was observed, with potential for the use as drug carriers [197]. Wang et al. used PVA microspheres as metallic nanoparticles carrier that could be used as embolic agents in transcatheter arterial embolization (TAE) therapy and also exhibited optimal magneto-thermal effect [198]. Wang et al. further investigated PVA microspheres use as drug carrier with a high adsorption capacity for small-molecule drugs [199]. In 2022, Yang et al. achieved chemical crosslinking of PVA microspheres using NaOH to obtain alkaline condition and then using boric acid as crosslinker; highly drug-loading capacity was achieved for DOX and embolization potential was demonstrated with the aid of a 3D printed embolization chip [200]. In a follow-up study in 2023 the performances of the previously obtained PVA microspheres were compared with similar PLGA, alginate and chitosan microspheres, demonstrating better blocking effects due to their higher elasticity and lower surface adhesion [201].

3.5.3. Polyacrylamide (PAAm)

PAAm is a non-degradable hydrogel used for mechanobiology, protein analysis, drug deliver and 3D cell culture. It is used for droplet generation thanks to its high stability and tunability of mechanical properties [202]. It is also used in cell behavior studies where fine control of mechanical and biochemical cues is needed, generally as a 2D substrate [203]. Earlier work by Seiffert et al. (2010) demonstrated the possibility to crosslink a modified PAAm, poly(N-isopropylacrylamide) (PNIPAAm), obtaining monodispersed droplets that could be tuned in size and morphology [202] (See Fig. 14A). The same research group then developed core-shell microcapsules with a PAAm core and a PNIPAAm shell achieving control over shell thickness and achieving multi-core microcapsules [204] (See Fig. 14C). PAAm was later used as a non-thermoresponsive shell whereas PNIPAAm as a thermoresponsive core to tune microparticles elasticity via core-deswelling, achieving desirable tuning properties for mechano-responsive scaffolds [205] Pure PAAm microspheres were produced by Yang et al. in 2012, through a thermal-initiated polymerization in n-octane at high temperatures (≧ 95 °C), obtaining fast polymerization time and good monodispersity [206]. In 2020, Wang et al. developed dissolvable PAAm microspheres by crosslinking acrylamide monomers with di-sulfide bridges using N, N'Bis(acryloyl) cystamine as crosslinker for high-throughput droplet DNA barcoding [207]. Finally, in 2023, Vigogne et al. combined parallelized emulsion formation and sequential droplet splitting in microfluidic devices, achieving much higher production rates than traditional single-channel or single-step devices paving the way for PAAm

microsphere mass-production [208] (See Fig. 14B).

4. Applications of DBM-engineered biomaterials in biomedical research

Droplet-based microfluidics enables the precise fabrication of biomaterials with well-defined size, morphology, and composition, including hydrogel microbeads, core-shell particles, microfibers, and nanostructures. These materials, engineered via DBM platforms using natural and synthetic polymers, provide biocompatible and tunable microenvironments that can interface with biological systems in highly controlled ways. In this section, we focus on the biomedical applications of such DBM-engineered biomaterials, rather than the microfluidic systems themselves. These include hydrogel microcarriers for drug screening and targeted delivery, 3D scaffolds for cell encapsulation and culture, and structured particles for single-cell encapsulation, detection, and sequencing. Additionally, DBM-fabricated materials have shown promise in high-throughput molecular evolution platforms, where precise compartmentalization and stability are critical. By centering on the functional use of biomaterials fabricated through DBM, we highlight how structural design at the microscale enhances their translational potential across various biomedical fields.

4.1. Drug screening and delivery with structured particles

Droplet-based microfluidics enables the fabrication of drug delivery systems with high precision in structure and composition [14]. Within this framework, biomaterials are not mere carriers: they actively shape pharmacokinetic behavior through properties like crosslinking, pH responsiveness, and permeability. This section analyzes four critical pillars of drug delivery—release kinetics, cytotoxicity and biocompatibility (IC50, hemolysis, and related assays), and drug distribution and loading efficiency — highlighting how biomaterial selection drives each outcome. While many DBM studies explore these axes, we focus here on illustrative examples to show how biomaterial design governs both efficacy and biocompatibility.

4.1.1. Kinetic release: modulation through shell composition and pH sensitivity

Biomaterial-driven control over drug release rates is a hallmark of DBM systems. The physicochemical properties of the employed biomaterials—such as crosslinking density, swelling behavior, porosity, and ionic responsiveness—determine how the encapsulated drug diffuses through the matrix or shell. These parameters can be finely tuned to achieve controlled, often pH-dependent, release profiles. The kinetics of drug diffusion from DBM-fabricated carriers are typically interpreted through classical models that describe the prevailing transport mechanisms (Table 7). Zero-order release implies a constant rate of drug delivery over time, a highly desirable but rarely achieved condition, typically requiring dense, non-degradable shells or reservoir systems. First-order kinetics follow an exponential decay, common in matrices where drug release is directly proportional to concentration. Second-order kinetics describe more complex systems, often involving cooperative interactions or autocatalytic degradation. The Higuchi model captures drug diffusion from porous matrices, where the cumulative release is proportional to the square root of time. Finally, the Korsmeyer–Peppas model is applied when the release mechanism is not purely Fickian but includes both diffusion and matrix relaxation or erosion—an effect frequently observed in responsive hydrogel systems produced via DBM.

Table 7
The equations and linear correlation of zero order, first order, second order, Higuchi, and Korsmeyer-Peppas model.

Kinetic model	Rate law	Linear correlation
Zero order	$[c] = -kt + B$	$[c] \sim t$
First order	$\ln[c] = -kt + B$	$\ln[c] \sim t$
Second order	$\frac{1}{[c]} = kt + B$	$\frac{1}{[c]} \sim t$
Higuchi	$[c] = kt^{1/2} + B$	$[c] \sim t^{1/2}$
Korsmeyer–peppas	$[c] = kt^n$	$\ln[c] \sim \ln t$

These models are not interchangeable, and the selection of the appropriate fitting curve reflects the interplay between biomaterial chemistry and release environment, emphasizing how material design governs the pharmacokinetics of the encapsulated drug. A representative case is provided by Yang et al. [209], who leveraged the complementary properties of alginate and chitosan to fabricate magnetically responsive microparticles for oral drug delivery using a simple L-junction DBM platform. In their design, alginate was used as the core matrix due to its mild ionic gelation and pH-sensitive swelling behavior, while chitosan served as a cationic coating to enhance stability and regulate permeability. The resulting microgels, obtained via a two-step crosslinking (pre-gelation in-channel and final ionic gelation in CaCl2), included Magnetic Alginate Microparticels (MAM) chitosan-coated MAM and(CMAM) with precisely controlled diameters (220–1000 μm). This biomaterial pairing was central to modulating the drug release profile: alginate conferred pH-responsiveness, with negligible drug diffusion at pH 2.1 (gastric environment) and rapid release at pH 7.4 (intestinal conditions), while the chitosan shell mitigated burst release, acting as a diffusion barrier and extending release duration. Notably, encapsulation efficiency rose from 75 % in MAM to 89 % in CMAM, and drug loading increased from 38 to 47 mg/g. The cumulative release profiles revealed a marked burst release in uncoated MAM, especially under physiological pH, whereas CMAM achieved a more gradual and sustained release (Fig. 15A). Importantly, kinetic modeling demonstrated that CMAM release followed the Korsmeyer–Peppas ($R^2 = 0.963$) and Higuchi ($R^2 = 0.961$) models, indicative of a diffusion-limited, non-Fickian mechanism, rather than ideal zero-order kinetics, which imply a constant release rate over time (see Fig. 15B and C). The observed trend—a rapid initial phase followed by a slower, diffusion-controlled stage underscores how the rational selection and pairing of biomaterials within DBM systems can overcome burst release while enabling programmable, pH-sensitive delivery. This study exemplifies how DBM not only offers physical control over size and shape, but—crucially—provides a platform to harness the functional chemistry of biomaterials for advanced drug delivery applications, directly linking material design to pharmacokinetic behavior.

4.1.2. Cytotoxicity and biocompatibility

In drug delivery systems fabricated via droplet-based microfluidics (DBM), the biocompatibility of the final carriers is intrinsically tied to the chemistry, crosslinking behavior, and purification of the biomaterials employed. Beyond ensuring effective drug release, carriers must minimize adverse biological responses, including cytotoxicity or inflammatory signaling, all of which are strongly influenced by material composition and residual reactants.

4.1.2.1. Carrier-dependent modulation of cytotoxic responses. Cytotoxicity represents a fundamental parameter in the evaluation of drug effectiveness, as it directly reflects their ability to damage cell viability and function *in vitro* [211]. Yet, this response is not dictated solely by the pharmacological activity of the encapsulated compound but is often critically shaped by the carrier matrix itself: residual monomers, crosslinkers, or degradation byproducts released from incompletely polymerized networks have been shown to drive dose-dependent toxic effects [212,213]. A comprehensive example is provided by Khan et al. [210], who used a coaxial DBM device to produce monodisperse core–shell microparticles via UV-initiated free radical polymerization. The core, composed of poly(methyl acrylate) (PMA), encapsulated ketoprofen, while the shell was made of PAAm or, in enhanced versions, poly

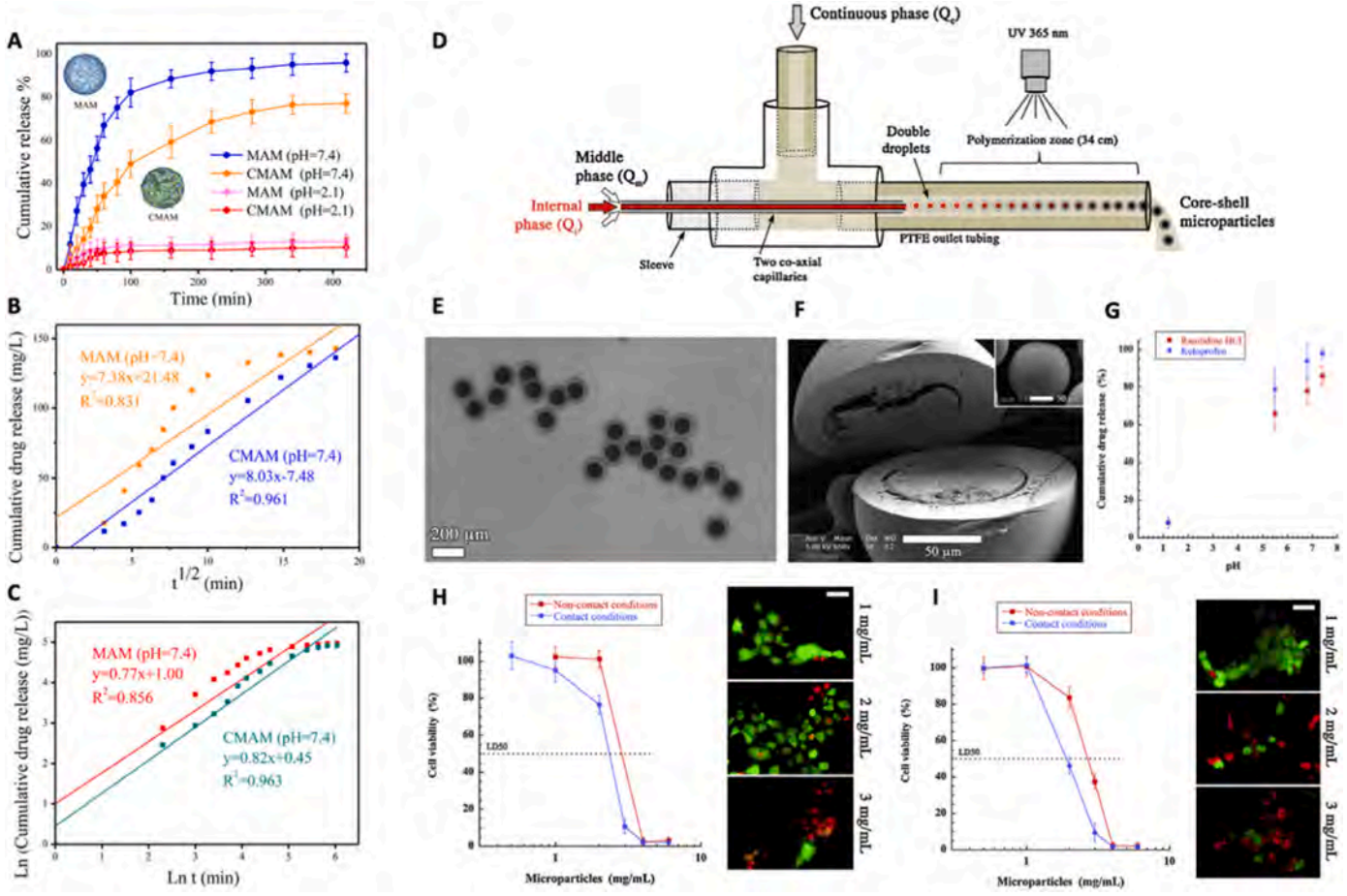

Fig. 15. Drug release and biocompatibility profiles of DBM-fabricated biomaterial-based microparticles. (A–C) Controlled release of amoxicillin from magnetic alginate microparticles (MAM) and chitosan-coated variants (CMAM), showing the impact of pH and particle size on cumulative release (A), Higuchi (B), and Korsmeyer–Peppas (C) kinetic modeling. Chitosan coating delayed diffusion and reduced burst release [209]. (D) Schematic of a two-coaxial capillary DBM system used by Khan et al. for producing core–shell microparticles via UV-polymerization. (E–F) Bright-field (E) and SEM (F) images showing spherical core–shell morphology with smooth acrylamide shell and poly(methyl acrylate) core (Scale bars: 200 µm). (G) pH-sensitive dual-drug release of ranitidine HCl (hydrophilic) and ketoprofen (hydrophobic), consistent with polymeric distribution and solubility behavior. (H–I) Cell viability as a function of microparticle dose under contact and non-contact conditions. Fluorescence micrographs show dose-dependent cytotoxicity and confirm high biocompatibility of optimized shell formulations (IC50 = 3.1 mg/mL). Thicker shells (higher Qm/Qi ratio) slightly reduced IC50 (2.7 mg/mL), likely due to residual monomer diffusion (Scale bars: 50µm.) [210].

(acrylamide-co-carboxyethyl acrylate) (poly(AMco-CEA)) for pH-responsive behavior (Fig. 15D–F). Drug release studies revealed a dual, selective release behavior: ranitidine HCl, being hydrophilic, was preferentially released at acidic pH, while the more hydrophobic ketoprofen exhibited accelerated release at neutral-to-basic pH (Fig. 15 G). This behavior was attributed to the differential swelling and permeability of the shell matrix, governed by biomaterial chemistry. The carboxyethyl acrylate units within the shell remained collapsed at low pH but ionized and expanded at higher pH, enhancing drug diffusion. This strategy enabled site-specific delivery, particularly for colonic release in inflammatory or neoplastic conditions. From a cytocompatibility standpoint, the choice and treatment of the shell material proved critical. Acrylamide is a well-known neurotoxic monomer; while polymerized PAAm is considered inert, any residual monomers, crosslinking agents, or photoinitiator byproducts can significantly reduce the biocompatibility of the final carrier. Khan et al. systematically analyzed this through non-contact MTT assays, which avoid direct mechanical interference and isolate chemical cytotoxicity. Their results showed that the optimized formulation (C1), characterized by a thin, well-polymerized shell, exhibited a Lethal Dose 50 (LD50) of 3.1 mg/mL, confirming low cytotoxicity and effective monomer removal (Fig. 15H and I). However, when the middle to inner phase flow ratio (Qm/Qi) was increased—resulting in thicker shells—the LD50 value dropped to 2.7 mg/mL, a significant reduction in cell tolerance. This was attributed to a higher probability of trapping unreacted acrylamide or surfactants within the denser matrix. Thicker polymer layers increase diffusion paths and reduce monomer escape during purification, which can prolong the leaching of cytotoxic residues over time. This study elegantly demonstrates that biomaterial composition and processing parameters are not neutral technical choices, but rather active modulators of cell compatibility. In DBM systems, where UV curing or ionic gelation is used for in situ polymerization, residual reactivity, crosslinking density, and functional group accessibility all shape the biological response. Thus, LD50 becomes a biomaterial-dependent property, and optimizing polymer networks for both functionality and safety is essential when transitioning from bench to biomedical application.

4.1.2.2. Inflammatory signaling. While IC50 provides a quantitative measure of cytotoxicity at the cellular level, the evaluation of inflammatory signalling offers a broader perspective on the host response to DBM-fabricated carriers. The choice of biomaterial is decisive, as its intrinsic chemistry, crosslinking density, and purity directly influence immune pathways. Hydrophilic and purified matrices such as alginate, hyaluronic acid, and PEG are generally associated with minimal cytokine induction, whereas impurities (e.g., endotoxins in alginate), excessive stiffness (e.g., highly crosslinked GelMA), or positively

charged coatings (e.g., chitosan) can activate NF-κB signalling and enhance secretion of TNF-α, IL-1β, and IL-6. Within droplet-based microfluidics (DBM), the ability to engineer monodisperse carriers with tuneable porosity and surface functionality allows these biomaterial-dependent effects to be precisely exploited. A compelling demonstration was provided by Boesveld et al. [214], who fabricated water-swollen PEGbased microgels (25 μm) functionalized with anti--TNF-α antibodies. Here, the PEG network played a dual role: its hydrophilic and non-fouling nature minimized unspecific inflammatory activation, while its porous architecture enabled homogeneous antibody distribution and high antigen accessibility, confirmed via confocal microscopy and FLIMFRET. As a consequence, the microgels were able to scavenge up to 88 % of TNF-α at 2.5 μg/mL, preventing cytokine-mediated cytotoxicity in HT29 cells and reducing IL-8 and COX-2 expression. Moreover, when exposed to LPS-stimulated human macrophages, the PEG network provided sufficient accessibility to neutralize nearly all secreted TNF-α. This study illustrates how biomaterial selection—in this case, PEG—was not a neutral choice, but the central factor enabling both high therapeutic efficacy and low off-target inflammatory signaling in DBM-engineered systems.

4.1.3. *Drug distribution and loading efficiency (in vivo biodistribution map)*

A crucial aspect of drug delivery systems are not only how efficiently a therapeutic payload is encapsulated, but also where and how it is distributed in vivo after administration. Biodistribution mapping provides essential information on the fate of carriers across tissues and organs, directly impacting therapeutic efficacy, systemic clearance, and potential off-target effects [215]. In this context, the biomaterial composition of the carrier plays a decisive role: hydrophilic and stealth-like matrices such as PEG or GelMA may prolong local retention

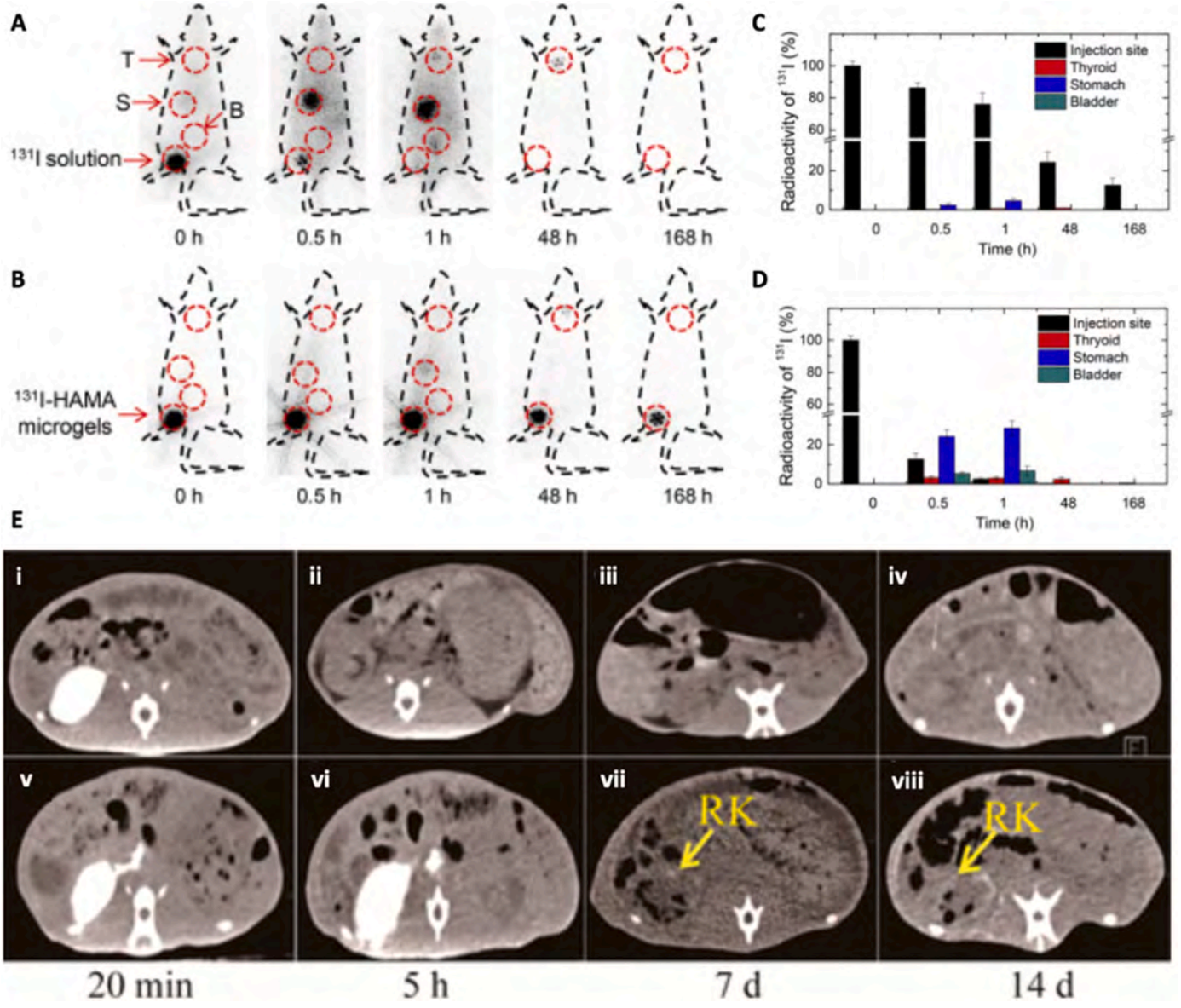

Fig. 16. In vivo biodistribution and imaging of DBM-fabricated biomaterial carriers. (A, B) Time-lapse scintigraphy images after intramuscular injection of (A)free ^{131}I solution and (B) ^{131}I-HAMA microgels in rats. Injection sites and organs of interest are indicated by red dotted circles (T: Thyroid, S: Stomach, B: Bladder). (C, D) Quantitative biodistribution plots of ^{131}I radioactivity normalized to the initial dose, showing rapid systemic clearance and off-target accumulation for free ^{131}I (C), versus sustained local retention with minimal leakage for ^{131}I-HAMA microgels (D) (n = 3) [217]. (E) CT scans of rabbits at different time intervals following embolization with KMG microspheres mixed with iodixanol (i–iv) or intrinsically radiopaque $BaSO_4$/ALG microspheres (v–viii). Unlike conventional KMG carriers, $BaSO_4$/ALG microspheres enabled long-term X-ray visibility of the embolized right kidney (RK), facilitating direct monitoring of embolization efficacy [218]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

or systemic circulation, while ionic or cationic polymers such as alginate or chitosan can enhance adhesion and tissue targeting but also accelerate clearance through immune recognition [216]. Droplet-based microfluidics (DBM) offers unique advantages for probing and optimizing these processes. By enabling the fabrication of monodisperse carriers with defined size, shell thickness, and spatial drug distribution, DBM provides a direct link between biomaterial-driven encapsulation efficiency (EE, the fraction of the initial drug dose successfully entrapped) and loading capacity (LC, the amount of drug loaded per unit weight of carrier), and the resulting in vivo biodistribution behavior. Moreover, the ability to incorporate imaging agents or functional fillers (e.g., $BaSO_4$, Fe_3O_4, perfluorocarbons) into microgels or microcapsules allows real-time mapping of carrier localization, bridging material design with pharmacological performance. A representative study exemplifying this principle was conducted by Kim et al. [217], who engineered ^{131}I-labeled photo-crosslinkable hyaluronic acid (HAMA) microgels using a droplet-based microfluidic generator. Hyaluronic acid was selected as the carrier matrix for its intrinsic biocompatibility and tissue affinity, while photo-crosslinking endowed the microgels with structural stability and predictable degradation kinetics. The DBM platform enabled the rapid fabrication of monodisperse, injectable radioactive biodegradable (IRB) microgels, ensuring both precise drug loading and uniform dose distribution. In vivo mapping through fluorescence tracing (FITC-HAMA) demonstrated stable localization of the microgels at the intramuscular injection site for over one week, with no inflammatory response. More critically, gamma scintigraphy and dynamic radioactivity scans (see Fig. 16A–D) revealed marked differences in biodistribution between free ^{131}I solution and ^{131}I-HAMA microgels. Free ^{131}I rapidly dispersed and accumulated in the thyroid, stomach, and bladder, whereas ^{131}I-HAMA microgels retained over 75 % of their activity at the injection site for 48 h, with residual radioactivity detectable beyond 3 weeks. Leakage to off-target organs was minimal (<1 % to the thyroid), confirming the ability of the HAMA biomaterial to confine and prolong therapeutic payload retention in vivo. This work highlights how the synergy between biomaterial chemistry (HA crosslinking) and DBM fabrication can transform drug distribution profiles, providing image-guided control over pharmacokinetics that is unattainable with free drugs or bulk hydrogels. Similarly, the development of $BaSO_4$--loaded alginate ($BaSO_4$/ALG) microspheres via droplet-based microfluidics for transcatheter arterial embolization (TAE), as outlined by Wang et al. [218], offers a particularly illustrative example. The use of alginate provides a safe and established matrix compared to synthetic embolic polymers such as PVA. Using a flow-focusing DBM device, droplets of sodium alginate premixed with Na_2SO_4 were generated in oil and collected in a $BaCl_2$ bath. Within each droplet, two reactions occurred simultaneously: ionic crosslinking of alginate with Ba2+ and in situ precipitation of $BaSO_4$ nanoparticles. This yielded monodisperse $BaSO_4$/ALG microspheres, where the radiopaque nanoparticles were uniformly embedded in the alginate network, providing intrinsic X-ray visibility without external contrast agents. Crucially, droplet microfluidics ensures that the microspheres are monodisperse, which is beneficial not only for reproducible embolization but also for predictable hemodynamic behavior and controlled drug release. The in vivo visibility of $BaSO_4$/ALG microspheres was evaluated in the renal artery of normal rabbits using digital subtraction angiography (DSA). Comparisons with commercial calcium alginate microspheres mixed with iodine-based contrast agents revealed the clear superiority of the $BaSO_4$/ALG formulation: while conventional alginate microspheres became invisible within hours (Fig. 16E(i-iv)), $BaSO_4$/ALG microspheres maintained high contrast visibility for up to 14 days (Fig. 16E(v-viii)), allowing direct monitoring of embolization efficacy and follow-up assessment of embolic sites. This example highlights how the integration of a radiopaque filler within a biocompatible alginate network—made possible by DBM precision—redefines both the biodistribution mapping and the therapeutic monitoring of embolic carriers.

4.2. Cell encapsulation and cultures via hydrogel-based droplets

Cell encapsulation is one of the most powerful applications of droplet-based microfluidics (DBM), and its success is largely dictated by the biomaterials used to construct the cellular microenvironment. Hydrogels such as alginate, GelMA, Matrigel, hyaluronic acid, PEG derivatives, and chitosan are widely employed, each imparting distinct physicochemical properties that regulate cell viability, proliferation, and differentiation. Parameters including ionic responsiveness, degradability, stiffness, and bioactivity determine not only the structural integrity of the capsule but also the type and quality of cell–matrix interactions it can support. DBM acts as an enabling platform by processing these biomaterials into monodisperse microgels or microcapsules with highly reproducible size, controlled porosity, and tuneable shell thickness. The synergy between material chemistry and microfluidic precision yields well-defined and isolated niches that can sustain either multicellular assemblies or single-cell encapsulation, depending on the intended application. Moreover, DBM highlights the decisive role of biomaterials in governing cellular outcomes: soft, ionically crosslinked matrices such as alginate favour high viability, whereas tuneable hydrogels like GelMA or PEG allow the modulation of stiffness and the presentation of biochemical cues that directly shape cell behavior. From this material-centric perspective, the choice of biomaterial—more than DBM itself—ultimately defines the biological relevance of encapsulated systems, shifting the paradigm from technical encapsulation toward the design of biofunctional microenvironments tailored for advanced cell culture and translational applications.

4.2.1. Biomaterial-driven morphogenesis in droplet-based 3D cultures

Within biomaterial-laden DBM systems, one of the most prominent outcomes is the generation of three-dimensional (3D) cell cultures, including spheroids and organoids [219]. In this context, biomaterials act not as passive scaffolds but as active regulators of tissue-like development, with their intrinsic chemistry and mechanics directly shaping cell aggregation, proliferation, and morphogenesis. Alginate, thanks to its mild ionic crosslinking and high cytocompatibility, has long been a preferred option in DBM-based encapsulation. Fang et al. demonstrated that mammary tumor tissues encapsulated in alginate microbeads developed both luminal- and solid-like organoid structures while preserving lineage heterogeneity and luminal progenitor populations with remarkable fidelity to the native tumor [220]. Yet, the same bioinert nature of alginate that safeguards tissue identity also constrains cell–matrix interactions, which in turn limits long-term proliferation and hampers the development of organized tissue architectures. This highlights alginate's limited capacity to support complex morphogenesis when used as a standalone material. To overcome such limitations, GelMA has emerged as a bioactive alternative, combining ease of processing with intrinsic adhesive motifs and tuneable stiffness. Although the use of bulk GelMA microgels has not yet been extensively explored in the DBM field, early attempts revealed structural drawbacks: compact and unstructured architectures constrained cell aggregation and impaired the formation of organized tissue assemblies. To address this, Wang et al. showed that processing GelMA into core–shell microgels via DBM—using a GelMA shell and a methylcellulose core—enabled enhanced proliferation, long-term viability, and even co-culture of hepatocytes and endothelial cells, highlighting the importance of structural design alongside material chemistry [85]. Beyond these, Matrigel and ECM-derived hydrogels remain powerful tools for organoid culture, offering complete repertoires of adhesion proteins and growth factors. Zhang et al. exploited DBM to generate cell-laden Matrigel droplets that rapidly yielded large, structurally organized lung tumor organoids with epithelial polarization within one week (see Fig. 17A and B), outperforming conventional batch cultures [221]. However, the temperature-sensitive sol–gel transition and batch variability of Matrigel limit its robustness and translational applicability in DBM workflows. Overall, each biomaterial presents unique strengths and

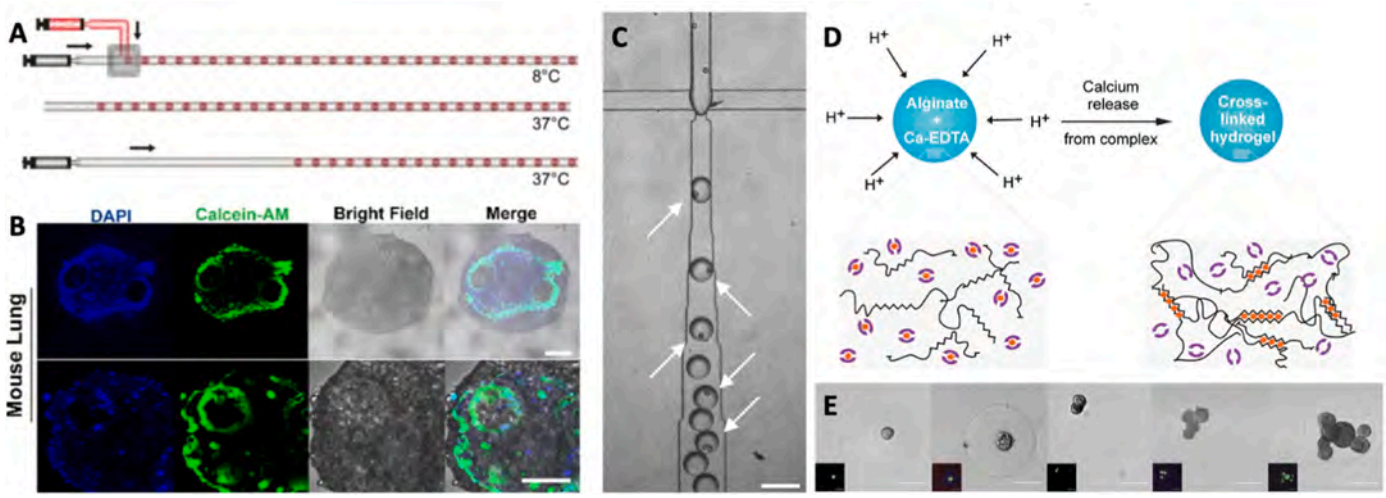

Fig. 17. Single-cell encapsulation and culture within hydrogel microenvironments using droplet-based microfluidics. (A) Schematic representation of Matrigel droplet generation: cells are suspended in cold Matrigel (8 °C) and emulsified into uniform droplets under co-flow with an oil phase in microfluidic tubing. After 30 min incubation at 37 °C, droplets solidify and are collected by infusing the tubing with oil. (B) Morphological characterization of mouse lung organoids derived from Matrigel droplets. Cells extracted from mouse lungs were seeded in Matrigel, and organoids were stained with DAPI (blue) and Calcein-AM (green). The circular fluorescence pattern indicates epithelial-like organization (Scale bar: 25 μm) [221]. (C) Microfluidic flow-focusing device for alginate droplet generation, with microscopic image showing single-cell–containing alginate droplets (white arrows) (Scale bar: 100 μm).(D) Schematic illustration of alginate crosslinking. Addition of acetic acid to the continuous phase dissociates the Ca-EDTA complex, releasing free calcium ions that induce controlled alginate gelation. (E) Representative images of cell-laden alginate microgels immediately after encapsulation and after 3, 6, 12, and 15 days of culture. Cells proliferated within the spherical microenvironments while maintaining viability, as confirmed by calcein staining (insets, confocal microscopy) (Scale bars: 25 μm) [225]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

weaknesses: alginate ensures mechanical stability, GelMA introduces tuneable bioactivity, Matrigel supplies rich biochemical cues, while others, such as hyaluronic acid, agarose, or chitosan bring complementary features. The distinctive contribution of DBM is the ability to harness and integrate these material-specific properties with precise control over droplet size, porosity, and architecture, ultimately creating microenvironments tailored for reproducible and physiologically relevant 3D cultures.

4.2.2. *Single-cell analysis enabled by DBM-generated carriers*

In addition to 3D multicellular systems, droplet-based microfluidics has also emerged as a cutting-edge technology for cell encapsulation at the single-cell level, offering the ability to confine single cells or small cell clusters within precisely controlled microenvironments [222]. This precision is particularly advantageous for applications requiring high-resolution cellular analysis, such as single-cell transcriptomics, drug screening, and protein secretion studies [36].

4.2.2.1. Single-cell cultures in hydrogel microcarriers. Encapsulating single cells within hydrogel droplets enables the isolation of individual cellular units in discrete, well-defined compartments, providing a powerful platform for investigating cell-specific behaviors, heterogeneity, and molecular responses [223]. Unlike systems designed for spheroid or organoid formation, single-cell encapsulation aims not at promoting aggregation, but at maintaining physical and functional separation between cells to allow high-resolution analysis. For an in-depth discussion of single-cell analysis using droplet-based microfluidics, we refer the reader to the comprehensive work edited by Lin et al. [224]. In this context, the choice of biomaterials plays a crucial role. Hydrogels such as alginate, agarose, chitosan, and collagen-based materials are particularly well-suited to the task, thanks to their biocompatibility, permeability, and tuneable crosslinking properties. These materials serve as aqueous precursors in which cells are suspended before emulsification in a water-in-oil system. Depending on the material and desired application, crosslinking may be achieved through ionic interactions, temperature shifts, or photopolymerization, resulting in the formation of stable microgels that preserve cell viability and functionality. Single-cell encapsulation in hydrogel droplets is particularly relevant for applications that require long-term monitoring of individual cells, such as studies of cell cycle dynamics, differentiation potential, and drug response. In these systems, each droplet acts as an independent microreactor in which a single cell can be cultured, observed, or manipulated without interference from neighbouring cells. This format enables the tracking of clonal proliferation, assessment of phenotypic variability, and acquisition of statistically robust data at the single-cell level — all within a scalable and controllable microfluidic framework. A representative study in this area was conducted by Utech et al., who introduced a method to finely regulate alginate gelation within droplets by employing a Ca^{2+}–EDTA complex that was dissociated via acetic acid in the oil phase [225]. By decoupling droplet formation from crosslinking, this strategy prevented clogging and enabled the fabrication of structurally homogeneous microgels (see Fig. 17C–E). From a biomaterial standpoint, the critical step was the functionalization of alginate with RGD motifs, which provided integrin-binding sites otherwise absent in the native polymer. This modification supported the adhesion and proliferation of encapsulated mesenchymal stem cells (MSCs), allowing long-term viability and growth for up to two weeks. Although this work demonstrated the promise of alginate as a biocompatible and tuneable hydrogel, it also illustrates a broader limitation: even when functionalized, alginate offers only a simplified approximation of the extracellular matrix, underscoring the need for more complex biomaterial strategies to better sustain single-cell function *in vitro*. In a related effort, Dolega et al. employed a flow-focusing droplet microfluidic system to generate Matrigel-based microbeads encapsulating single epithelial cells [165]. Within these confined microenvironments, individual prostate cells proliferated and differentiated into well-structured acini, each droplet acting as an independent 3D culture unit without interference from neighbouring cells. Compared to conventional bulk 3D culture, the approach produced acini with markedly more homogeneous size while maintaining comparable growth kinetics. Importantly, the microfluidic format enabled continuous monitoring of acinar development, capturing the entire process from the first cell division through to lumen formation and final morphogenesis. By

leveraging the bioactive composition of Matrigel, the authors provided a microenvironment rich in adhesion ligands and growth factors, thereby facilitating cell polarization and self-organization into physiologically relevant structures. Furthermore, a special mention should be made of poly(ethylene glycol) (PEG)-based hydrogels, which have gained prominence in single-cell encapsulation for their inert and highly tuneable chemistry. PEG offers a reproducible "blank-slate" matrix that can be functionalized with adhesion ligands or degradable linkers, making it attractive for applications such as single-cell transcriptomics and drug screening. Yet, PEG systems traditionally suffered from oxygen inhibition during photopolymerization, especially at the microscale, leading to incomplete gelation and reactive oxygen species (ROS) that compromise cell viability. A notable advance in overcoming these limitations was demonstrated by Si et al. [226], who designed a droplet microfluidic platform for deterministic single-cell encapsulation within PEG-norbornene (PEGNB) hydrogels. By combining inertial cell focusing with thiol–ene step-growth photopolymerization, they achieved >90 % viability of mesenchymal stromal cells (MSCs) over 7 days, alongside enhanced anti-inflammatory cytokine expression (IL-10, TGF-β). In vivo, encapsulated MSCs showed strong retention, reduced inflammation, and mitigated liver damage, underscoring PEGNB's translational potential for therapeutic single-cell encapsulation.

Collectively, these studies demonstrate how droplet-based microfluidics enables the isolation and long-term culture of single cells within tailored hydrogel microenvironments. By confining individual cells in defined biomaterial compartments, DBM systems make it possible to monitor proliferation, differentiation, and morphogenesis at the single-cell level with high fidelity and reproducibility. This shift from bulk to single-cell culture is crucial, as it allows the dissection of clonal behaviors and cell-to-cell variability that would otherwise be masked in heterogeneous populations. Beyond providing a controllable culture platform, single-cell encapsulation in DBM can be readily interfaced with advanced analytical methods such as mass spectrometry [227], flow cytometry [228], or rolling circle amplification (RCA) [229], enabling functional profiling of rare or clinically relevant cell types. In this way, the convergence of biomaterials and droplet technologies establishes a powerful framework for probing cellular heterogeneity and function one cell at a time, with direct implications for personalized medicine, early diagnostics, and regenerative therapies.

4.2.2.2. Single molecule detection using functional droplets. In addition to providing a highly controlled and isolated microenvironment for cell encapsulation and proliferation — down to the single-cell level — hydrogel-based droplet microfluidics also finds application in contexts that require highly sensitive and selective detection of individual biological entities. In the fields of chemistry, biology, medicine, and environmental science, there is an increasing demand for technologies capable of detecting single molecules — particularly nucleic acids and proteins — as well as individual cells with exceptional precision [222,230]. Within this framework, biomaterial-laden droplets play a crucial role: their sol–gel transition properties and tuneable porosity not only create stable reaction compartments, but also regulate diffusion and preserve enzymatic activity during in-droplet assays, thereby directly impacting the robustness and sensitivity of detection. These features are particularly advantageous for amplification strategies, where the interplay between hydrogel composition and biochemical processes can modulate signal quality and reproducibility. The detection of nucleic acids at the single-molecule level is of paramount importance for biomedical research, molecular diagnostics, and drug development [231]. Polymerase chain reaction (PCR) remains one of the most powerful technologies in this context due to its remarkable amplification capacity, enabling the generation of a large number of copies from a single sequence. Within droplet-based microfluidics, the incorporation of hydrogel matrices provides distinct advantages over conventional oil-only systems: by stabilizing droplets against thermal cycling, minimizing reagent loss, and limiting molecular diffusion, hydrogels improve amplification fidelity and support downstream analytical handling [232,233].

In this regard, Leng et al. developed a microfluidic platform based on agarose droplets for emulsion PCR, which proved highly efficient for detecting single copies of DNA [234]. The agarose-in-oil droplets were generated through a flow-focusing microfluidic chip at a frequency of approximately 500 Hz, each containing a single DNA template, an agarose solution, and the PCR reagent mix. Thanks to the thermoresponsive sol–gel transition of agarose, the droplets remained in the liquid phase throughout the thermal cycling, ensuring high amplification efficiency (95 %), and subsequently solidified upon cooling. This process preserved the monoclonality of the amplified DNA even after the oil phase was removed, making the system compatible with downstream analyses such as flow cytometry, DNA sequencing, or long-term storage. Another significant example is provided by the work of Tamminen et al., who proposed an emulsion-based procedure for encapsulating single microbial cells and amplifying their entire genome within multilayered hydrogel droplets [235]. In this architecture, microbial cells were initially trapped in rigid polyacrylamide droplets, which preserved genome integrity after cell lysis. These droplets were then converted into agarose-based picoliter reactors for the multiple displacement amplification (MDA) reaction. The amplified products were fluorescently labeled via PCR and analyzed by flow cytometry, enabling the detection of specific microbial genes even within complex populations. For instance, genomes of *E. coli* XL1 present at 0.1 % could be distinguished from *E. coli* MC1061, demonstrating the high sensitivity of the platform. Likewise, protein-level detection represents another emerging application, although historically more challenging than genetic analysis. Chokkalingam et al. employed an agarose droplet system to investigate the functional heterogeneity of stimulated Jurkat T cells, encapsulating single cells together with antibody-functionalized microbeads targeting IL-2, TNF-α, and IFN-γ [228]. During incubation, secreted cytokines were captured by the beads within the same droplet, which was then solidified by cooling. Following emulsion breaking, the captured proteins were detected via immunofluorescence and flow cytometry. This method allowed the observation of variability in cytokine secretion profiles, highlighting the effectiveness of droplet-based microfluidics in single-cell functional profiling.

Altogether, these studies demonstrate the potential of hydrogel-laden droplets to extend the scope of DBM beyond cell culture into the realm of highly sensitive molecular and protein detection. Such platforms enable not only the amplification of nucleic acids at the single-molecule level but also the functional analysis of secreted proteins from individual cells, thereby broadening the analytical reach of droplet microfluidics. To the best of our knowledge, polysaccharide-based hydrogels have so far remained the predominant choice in this domain, a reflection of their robustness and ease of use, but also an indication that the exploration of alternative hydrogel chemistries remains at an early stage.

4.2.2.3. Single-cell sequencing supported by DBM particles. Building upon the framework of single-cell analysis, droplet microfluidics based on hydrogels has recently gained momentum in the field of single-cell sequencing — a frontier area for investigating genetic expression variability among individual cells [236–238]. This approach has proven particularly valuable in the analysis of rare and heterogeneous cell populations, such as circulating tumor cells, prenatal samples, and early-stage embryonic cells [239,240]. Despite its potential, single-cell genomic sequencing presents significant technical challenges, including time-intensive parallel processing, inefficient handling of minute quantities of starting material, and amplification biases arising from low DNA input. Hydrogel droplet microfluidics has emerged as a powerful tool to overcome these limitations. In addition to offering high-throughput capability, low reagent consumption, and simplified

manipulation, hydrogel droplets with three-dimensional polymer networks provide mechanical support for maintaining the integrity of lysed-cell genetic content while still allowing access to amplification reagents. Novak et al. introduced a robust agarose droplet platform for multiplexed genetic detection and sequencing at the single-cell level [241]. In their system, individual cells were encapsulated in agarose droplets containing primer-functionalized microbeads. After solidification, the beads underwent cell lysis, releasing genomic DNA that was retained within the agarose matrix due to its small pore size (1.5 %). Following equilibration with a PCR mixture, the droplets were re-emulsified to carry out parallel single-cell PCR reactions, enabling downstream genotyping and expression analysis. The same group later extended the method to single-cell forensic short tandem repeat (STR) profiling. Using multiplexed droplet PCR and capillary electrophoresis, STR targets were transferred to capture beads, enabling fragment analysis with high fidelity. The agarose hydrogel matrix ensured consistent cell lysis, DNA retention, and controlled amplification, establishing a reliable platform for singlecell genomics, rare mutation detection, and forensic investigation. Complementing this, Bigdeli et al. developed an alginate-based droplet system for single-cell whole genome amplification (WGA) [242]. In their method, cells suspended in alginate solution were encapsulated via droplet spraying, followed by cell lysis and two-step WGA within the hydrogel matrix. The high-molecular-weight DNA products were retained within the alginate beads, minimizing cross-contamination and maintaining compartmentalization. After extraction, the DNA was sequenced using the Illumina MiSeq platform, with results showing high concordance with the NCBI reference database. The authors noted that the platform could benefit further from optimized amplification protocols, such as MALBAC, to achieve improved genome coverage and uniformity.

4.3. *Molecular evolution using encapsulated Libraries in DBM microgels*

Droplet-based microfluidics has proven to be a powerful platform for *in vitro* molecular evolution, particularly for the high-throughput selection of functional nucleic acids. By compartmentalizing individual DNA molecules within hydrogel droplets, this approach ensures monoclonality and preserves the genotype–phenotype linkage essential for selection. A representative example is the work by Zhang et al., who employed agarose droplet microfluidics to accelerate the SELEX (Systematic Evolution of Ligands by EXponential enrichment) process for aptamer discovery [243]. Single DNA templates from a pre-enriched ssDNA library were encapsulated into uniform agarose droplets and subjected to emulsion PCR. After solidification, the resulting monoclonal beads were screened by flow cytometry for binding affinity to the target protein Shp2. Only droplets containing high-affinity binders were recovered for sequencing, significantly streamlining the aptamer selection process. This method combines the precision of microfluidic compartmentalization with the efficiency of high-throughput screening, offering a cost-effective alternative to conventional SELEX.

5. Conclusion

Droplet-based microfluidics has established itself as a cutting-edge platform for the precise manipulation of biomaterials, offering unprecedented control over the generation of architecturally defined microsystems for biomedical applications. Central to this technological advancement is the integration of natural, semi-synthetic, and synthetic hydrogels, which serve not only as structural matrices but also as dynamic functional components capable of responding to biological cues. These biomaterials enable the fabrication of highly tunable carriers—such as nanoparticles, microparticles, core–shell microcapsules, and microfibers—each with specific mechanical, biochemical, and diffusional properties tailored for drug delivery, 3D cell culture, tissue modeling, and single-cell analysis. By leveraging the unique capabilities of DBM to modulate droplet size, internal morphology, and gelation mechanisms with high precision, it is now possible to design microenvironments that closely mimic the extracellular matrix, sustain cell viability, and facilitate controlled therapeutic release. This synergy between microfluidic engineering and biomaterial science has led to new strategies for constructing high-throughput and personalized platforms with improved reproducibility, scalability, and biological relevance.

Looking ahead, several application niches are poised for rapid growth. Intra-tumoral immunomodulation can exploit injectable, DBM-fabricated microgels as local depots for spatiotemporally controlled delivery—or sequestration—of cytokines, chemokines, and checkpoint modulators, thereby shaping immune gradients while minimizing systemic exposure [214,244]. Point-of-care diagnostics stand to benefit from dropletized assays with single-molecule sensitivity integrated into robust, sample-in/answer-out cartridges; DBM monodispersity enhances analytical precision, reduces reagent consumption, and shortens time-to-result [245]. Perhaps most transformative, organoid-based personalized therapy can leverage DBM-templated, biomaterial-laden microenvironments as a patient-specific biological twin: microbead scaffolds that preserve intra-organoid heterogeneity yet ensure inter-patient comparability through standardized fabrication, enabling rapid and cost-aware testing of drug combinations and dosing strategies [246].

Despite these achievements, several challenges remain, particularly in optimizing gelation kinetics compatible with live cells, integrating real-time analysis modules, and bridging the gap between proof-of-concept systems and clinically viable products.

To translate DBM platforms from benchtop prototypes to clinically approved devices regulatory hurdles, scaling up manifacturing and taking into account of cost-effectiveness must be addressed. From the regulatory standpoint, both Food and Drug Administation (FDA) and European Medicines Agency (EMA) lacks specific guidelines dedicated to DBM platforms. The Center for Devices and Radiological Health (CDRH), one of the six products center of FDA has established a Microfluidics Program to conduct regulatory science research on DBM platforms, focusing on fluid flow issues, materials, and manufacturing methods standardization both for safety and consistency of the devices and performance testing protocols [247]. On the other side, EMA is addressing the regulatory issues by integrating this technology within the broader context of advanced therapy medicinal products (ATMPs), considering DBM platforms as a part of the overall manifacturing and quality control strategy [248].

Scaling up manufacturing of DBM platforms has also to take into account of the mismatch between materials that can be used for prototyping and materials that can be used on an industrial level. PDMS and soft lithography approaches are the most used at a laboratory scale, however PDMS presents problems for clinical products such as absorption of small molecules, gas permeability, unstable surface chemistry and difficulty in reproducible manufacturing [249]. To scale up the production of such devices, a change of direction in the materials used for the chips is needed, as injection-molding of thermoplastic material is the most reliable for process validation. Parallelizing prototyping with manufacturability engineering would shorten the times required for clinical translation.

Moreover, manufacturing system must address several requirements such as a proven Quality Management System (QMS, ISO13485), design history files (DHF), risk management (ISO 14971), supplier controls and traceability that are currently lacking in laboratory-scaled prototypes [250].

Finally, translation to clinic must take into account of cost-effectiveness analyses, adopting a clear cost-benefit model that is highly demanded by healthcare provided, promoting the high-throughput capabilities and potentiality of miniaturized, low-volume assays that are key to demonstrating the economic values of such devices [13].

Addressing these issues will require further interdisciplinary efforts at the interface of materials science, bioengineering and translational

medicine as well as expertise in regulatory affairs and manufacturing engineering.". Ultimately, the convergence of DBM and advanced biomaterials is shaping a new paradigm in biofabrication, enabling the creation of miniaturized, functional, and patient-specific systems that hold great promise for regenerative medicine, precision therapeutics, and next-generation biological research.

CRediT authorship contribution statement

Andrea Fergola: Writing – original draft, Conceptualization. **Cesare Gabriele Gaglio:** Writing – original draft, Conceptualization. **Simone Luigi Marasso:** Visualization, Supervision. **Matteo Cocuzza:** Writing – review & editing, Supervision. **Candido Fabrizio Pirri:** Supervision, Project administration, Funding acquisition. **Lucia Napione:** Writing – review & editing, Conceptualization. **Francesca Frascella:** Writing – review & editing, Supervision.

Declaration of competing interest

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

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Data availability

No data was used for the research described in the article.

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