Core-Shell Microcapsules: Biofabrication and Potential Applications in Tissue Engineering and Regenerative Medicine

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Abstract

The construction of biomaterial scaffolds that accurately recreate the architecture of living tissues *in vitro* is a major challenge in the field of tissue engineering and regenerative medicine. Core-shell microcapsules hold great potential in this regard, as they can recreate the hierarchical structure present in most biological systems. The independent modulation of the composition of both core and shell layers allows the design of compartmentalized platforms tailored to the recreation of specific cell niches. Techniques such as superhydrophobic surfaces, microfluidics, electrospray, and layer-by-layer assembly have been successful in producing core-shell microcapsules for the encapsulation of cells and bioactive factors. This review provides an overview of available materials and techniques used in the generation of core-shell microcapsules, while also highlighting some of their potential applications in the design of innovative and effective tissue engineering and regenerative medicine strategies.

Keywords: Microencapsulation, microcapsules, core-shell structures, tissue engineering, regenerative medicine.

1. Introduction

Organ transplantation is, in many cases, the only viable option for the treatment of damaged organs, however, it is heavily limited by factors such as the reduced availability of donors and the rejection of transplants due to the body's immune response[1]. The search for alternative therapeutic options has led to an increased interest in the field of tissue engineering and regenerative medicine (TERM). TERM approaches seek to combine the use of cells, biomaterial scaffolds and bioactive molecules to promote tissue repair *in situ* and possibly replace damaged tissue[2,3]. These approaches can include the development of

bioengineered cells[4], microtissues[5], organoids[6] and potentially even whole organs[7], in order to produce biological structures for transplantation, as well as robust *in vitro* models of disease that can be implemented in the study of potential drug candidates. The culture, assembly and delivery of these structures can take advantage of microencapsulation strategies in order to produce closed scaffolds that maintain the viability of cells while isolating them from the surrounding environment.

Microencapsulation is a process used in a wide range of industries to preserve products of interest, such as essential oils[8], growth factors[9], drugs[10], proteins[11], bacterial cells[12] or mammalian cells[13] by enveloping them in a polymer coating. Originally proposed in 1964[14], microencapsulation has been used to preserve the stability of sensitive molecules during delivery and shield cells from the immune system, preventing their recognition and rejection, and forgoing the need for immunosupressants, which carry dangerous side effects[15,16].

Core-shell microcapsules consist of multicompartmentalized platforms in which one or more cores are enveloped by an outer layer, known as the shell. This shell provides mechanical stability to the microcapsules, regulates mass transfer and isolates the encapsulated components from the surrounding environment, while the core provides an enclosed space that should ensure the stability and viability of encapsulated molecules and cells, respectively. Because the core and shell are independent structures, their properties can be adjusted separately, resulting in highly tunable microencapsulation platforms that better reproduce the hierarchical and compartmentalized 3D architecture of natural systems[17,18].

In this review, advances in the development of core-shell microcapsules for application in biomedical applications will be examined. An overview of commonly used biomaterials in the construction of core-shell microcapsules will be presented, followed by an exploration of the most relevant biofabrication techniques. Subsequently, some current applications of microcapsules in the study and treatment of relevant disorders will be reviewed.

2. Architecture of core-shell microcapsules

2.1. Biomaterials used in the generation of the shell

The shell is a semipermeable coating that regulates mass transfer between the interior of a microcapsule and the surrounding environment. As such, it should allow the diffusion of small molecules, permitting the entry of nutrients and oxygen required for cell growth and the release of metabolic waste products or encapsulated drugs and bioactive factors. By

adjusting the characteristics of the polymer shell, such as its thickness and porosity, it is possible to control the release rate of these molecules, which is useful in the design of platforms for the sustained release of drugs, proteins and paracrine factors[19]. The shell also functions as a protective barrier, ensuring the mechanical stability and integrity of the capsules while shielding its contents from the outer environment and providing immunoprotection to the encapsulated materials.

Biomaterials used in the generation of the shell must be biocompatible, non-immunogenic and provide mechanical stability to the microcapsules. One of the most prominently used polymers in the biofabrication of microparticulate materials is alginate, which encompasses a family of negatively charged polysaccharides that can be obtained from brown algae, consisting of linear copolymers of (1,4)-linked β-*D*-mannuronate and α-*L*-guluronate [20]. These materials are generally non-thrombogenic, non-immunogenic, biocompatible and can be acquired for a low cost[21,22]. Alginates are optimal for the production of scaffolds in mild conditions, due to their ability to form hydrogels when placed in contact with a solution of divalent cations such as Ca^{2+}, Ba^{2+} or Sr^{2+}, through a process known as ionotropic gelation[20,23–25].

Alginate shell microcapsules have frequently been prepared through electrospray and microfluidics by coating the core material with an alginate solution and introducing a solution containing calcium ions, which crosslinks the alginate[26]. Reversely, it is also possible to introduce droplets of a solution containing Ca^{2+} into an alginate solution, producing capsules with a thin membrane and a liquid core[27]. The addition of surfactants to the alginate solution has been reported to allow the encapsulation of a wide variety of liquids within microcapsules with thin shells[26]. Alginate shell-PLGA core microcapsules have been successfully used in the controlled delivery of bioactive molecules, including metal ions, drugs and growth factors[28–30]. Alginate has also frequently been combined with different polycations in layer-by-layer (LbL) assembly to produce microcapsules with multilayered shells[31–33].

Despite its beneficial properties and widespread application, alginate also presents significant drawbacks. The relative content of each alginate in mannuronic acid and glucuronic acid influences its mechanical properties, stability and permeability, which impacts the reproducibility of biofabrication methods. It has also been suggested that alginates with a high content in mannuronic acid may increase the likelihood of triggering

an inflammatory response[34–36]. Due to its natural source, alginates used in biomedical applications must undergo an extensive purification procedure, in order to fully remove contaminants that will reduce their biocompatibility, such as polyphenols, proteins or endotoxins, which increases its cost[20,35,36]. Furthermore, alginate does not naturally possess domains for cell adhesion, which results in low cellular attachment to the resulting hydrogels[37], however, it has been shown that cell adhesion can be improved by introducing ECM-derived peptide moieties in the polymer backbone, such as RGD[21,38], YIGSR[39] or DGEA[38].

While the ionotropic gelation of alginate is a commonly pursued strategy to produce both the shell and core of the microcapsules, another commonly employed method is the use of polymers containing methacryloyl moieties. These functional groups allow the production of hydrogels through photopolymerization, a process that can also be performed in mild conditions. This strategy has been employed to produce microcapsules for cell encapsulation and drug delivery using microfluidic platforms[40,41] as well as superhydrophobic surfaces[42]. One of the most prominently used photocrosslinkable polymers is gelatin-methacryloyl (GelMA), a chemically modified form of gelatin[43]. Gelatin is a natural, non-immunogenic, biocompatible and biodegradable polymer with relatively high solubility, which possesses bioactive motifs that promote cell adhesion[44,45]. While gelatin solutions can be thermally crosslinked, the introduction of methacryloyl moieties can be used to produce hydrogels with improved mechanical properties, which can be modulated by adjusting the substitution rate of methacryloyl groups or the polymer density[46]. Recently, the modification of gelatin with catechol-like moieties has also been reported[47]. This strategy allows the production of entirely protein-derived systems through coordination with iron, generating a robust shell with adhesive properties that induces cell attachment to the inner shell wall, promoting the formation of a cell monolayer along its curvature.

Microcapsule shells have also been prepared using inorganic molecules. Cha et al. reported the formation of a silica hydrogel to coat cell-laden GelMA microcarriers[48]. Cardiac cells were cultured on the surface of spherical GelMA microgels produced in a microfluidic platform. The silica shell was then introduced by a sol-gel procedure to protect the cells from mechanical stress, oxidative pressure and exposure to immune cells. The shell was shown to protect the encapsulated shells from highly oxidative agents without compromising cell migration or proliferation. Furthermore, the silica shell was shown to be biodegradable,

producing metabolites that are safely excreted from the body. Alginate core silicate shell microcapsules have also been developed for bone TERM applications[49]. The capsules were shown to induce the formation of apatite *in vitro* when placed in simulated body fluid. Additionally, they were also shown to be an efficient carrier for the sustained release of proteins, achieving high protein loading efficiency.

Synthetic polymers have also been proposed as substitutes for alginate and other natural materials, as they are highly tunable materials that can be produced in a reproducible manner while also possessing improved mechanical properties[50]. One such polymer is poly(ethylene glycol) (PEG), which has prominently been used in cell encapsulation[4,51,52]. It is both biocompatible and highly bioinert, although it can be functionalized to introduce hydrolysable segments, bind growth factors and introduce chemical groups that improve cell adhesion and modulate the immune response[53–55]. As such, PEG is a compound of great interest in the generation of core-shell capsules for cell encapsulation and delivery of bioactive factors. For example, dithiothreitol-modified PEG-diacrylate has been used to encapsulate heparin microparticles, producing core-shell structures with biodegradable shells and multiple cores for the sequestration, isolation and delivery of proteins[56]. These capsules provide a system that preserves the structure and activity of growth factors while also offering tight control over the timeframe of their release[56]. PEG has also been explored in the design of aqueous biphasic systems for generation of microcapsules in oil-free microfluidic platforms[57], which will be discussed in greater detail in a later section of this review.

2.2. Core structure

In cell encapsulation, the role of the core is to provide appropriate conditions for continued cell survival and proliferation[58]. In order to guarantee a suitable microenvironment for the growth of encapsulated cells, the cores of the microcapsules should mimic relevant properties of native tissues. Hydrogels have typically been used in the production of microcapsules and other biomaterial scaffolds, as they consist of highly hydrated materials organized in porous structures possessing mechanical properties that mimic those of soft tissues and the native extracellular matrix (ECM)[58–61]. As such, hydrogels derived from natural tissues have often been incorporated in the core of the microcapsules, producing matrix-core microcapsules. Cell encapsulation in core-shell microcapsules has included

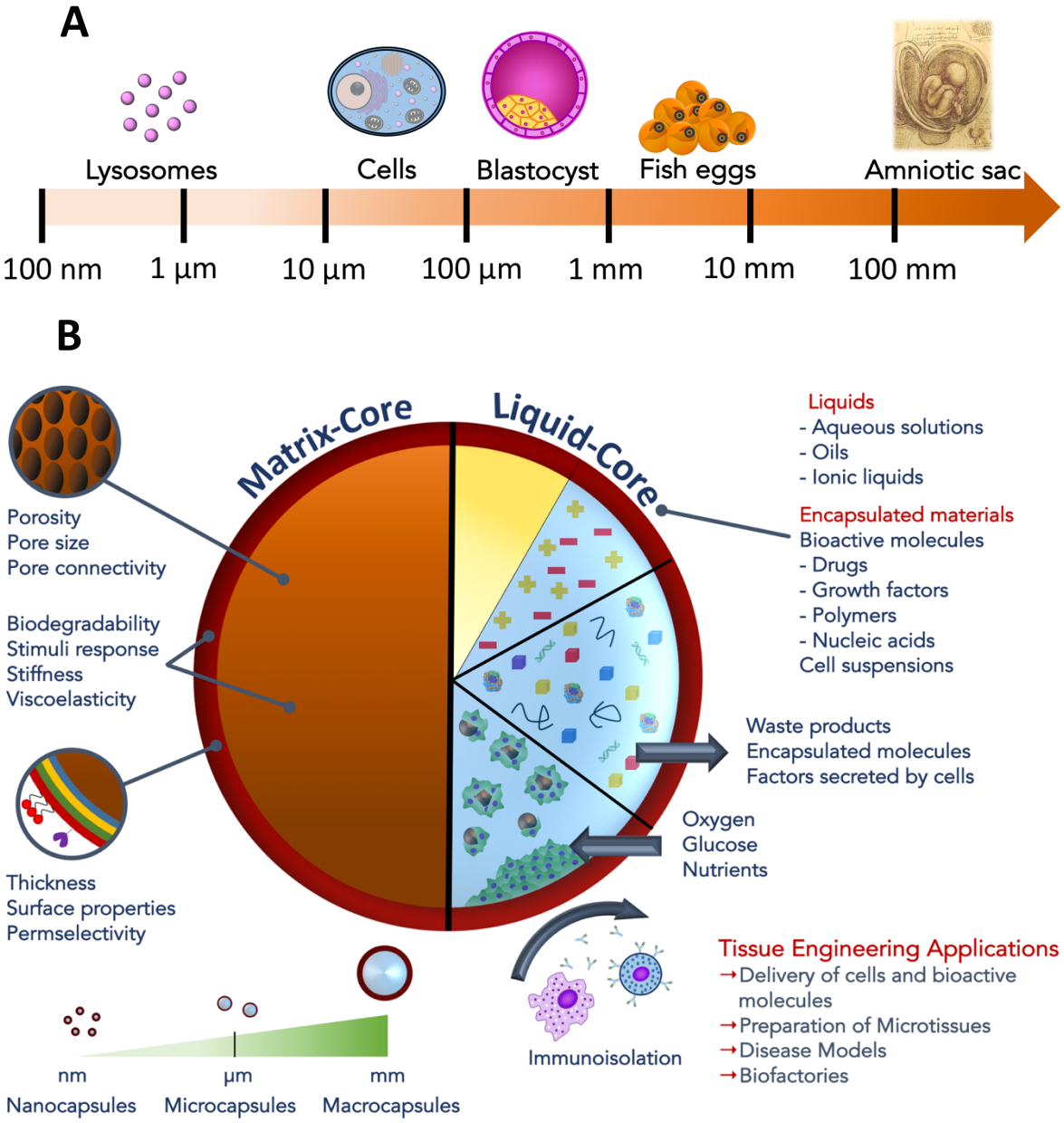

Fig. 1. Schematic representation of core-shell systems. (A) Liquefied core-shell structures are universally present in biological systems across multiple scales; (B) The properties of core-shell microcapsules platforms can be tailored toward different applications, providing a highly versatile platform for TERM approaches.

materials such as Matrigel®[62], collagen I[63], GelMA[48], or decellularized ECM[64], which act as support systems for the proliferation of cells, tissues and organoids[62].

While these porous hydrogel scaffolds have been prominently used in the encapsulation of cells and proteins, the polymer matrix heavily restricts the movement of cells as well as the diffusion of nutrients and oxygen required for their survival. As such, a possible alternative

has emerged. By taking inspiration from biological structures (Figure 1A) such as fish-eggs[26] and embryo[65], liquid core capsules, in which the internal core is in the liquid state, can achieve enhanced mass transfer while also allowing the free movement of cells in the aqueous environment of the core, improving cell-cell interactions and allowing the cells to self-organize into more complex 3D structures, such as cell spheroids[66–68]. A wide variety of liquids can be incorporated in the cores of the microcapsules, including oils[69] or even ionic liquids, which could have applications in areas such as chemical catalysis, production of pharmaceuticals and the remediation of polluted environments[70].

Liquid core microcapsules with alginate shells have been proposed as aqueous bioinspired 3D platforms for the culture of embryonic stem cells (ESCs), which can be differentiated into different cell lines for cell replacement therapy[26,65,71]. Before encapsulation, cells are usually suspended in culture medium, a buffer solution, saline or a mannitol solution, which is then incorporated in the core of the microcapsules[71,72]. These capsule-based systems have been shown to improve the stability of long-term *in vitro* cell culture when compared to cell culture in solid beads[67]. The suspension of cells in a polymer solution is also a possibility to modulate the characteristics of the core and provide cues that can modulate cell behavior. For example, Park et al. utilized hyaluronic acid/alginate core-shell capsules to produce MSC spheroids with the purpose of promoting angiogenesis *in vivo*[68]. Alternatively, it is possible to obtain liquid core capsules for cell encapsulation by entrapping the cells in a polymer particle, which is then used as a template for the deposition of a multilayer membrane, which will constitute the shell. The template core is then liquefied. This was the basis of the first cell microencapsulation procedure, performed by Lim and Sun[33], who encapsulated pancreatic islets in a core-shell capsule with a liquefied core of alginate. This process involves the use of chelating agents, such as EDTA or sodium citrate[73,74], which sequester the divalent cations responsible for the ionic crosslinks in the alginate hydrogels. Gelatin can also be used in the formation of liquefied core capsules due to its mechanism of thermal gelation. Gelatin solutions produce hydrogels when cooled at low temperatures, while higher temperatures result in the liquefaction of the hydrogels[75]. As such, it is possible to produce gelatin microparticles by cooling droplets of a gelatin solution at 4°C, which can then be coated with an outer membrane[75,76]. The gelatin cores return to the liquid state at physiological temperature, resulting in liquefied cores after implantation.

A considerable limitation of liquid core microcapsules in cell encapsulation is the inability of cells to survive in suspension. In the absence of a suitable substrate for cell adhesion, most mammalian cell lines initiate a form of programmed cell death known as anoikis. In living tissues, this process guarantees that only cells that are successfully integrated into the tissue can survive[77–79]. The anchorage-dependent character of these cells thus requires the addition of other structures that permit cell attachment. Although certain microcapsule-based platforms allow cell to attach to the inner wall of the shell[47], the most commonly used strategy to overcome the anchorage-dependence of mammalian cell lines is the co-encapsulation of cells with microcarriers[80,81]. These microcarriers consist of polymeric microparticles that provide cells with a surface for attachment, and which can be tailored to present cells with mechanical and structural cues to guide their proliferation, differentiation, orientation and aggregation. A more in-depth look into the features of these structures will be provided in the following section.

2.3. Microcarriers

The use of microcarriers as attachment sites in suspension culture was originally proposed by van Wezel in 1967, providing a 3D platform that allows the culture of cells in bioreactors[82]. Microcarriers have since been used as platforms for cell therapy and tissue engineering[83–86] and the production of recombinant factors[87], viral vaccines[88–90] and more recently, synthetic meat[91].

2.3.1. Structure of the microcarriers

Microcarriers used in cell culture have displayed spherical, cylindrical, hexagonal, disk-like and lens-shaped geometries[92–95]. The geometry of the microcarriers, combined with the topographical features of their surface, influences the organization and orientation of attached cells, as well as the available surface area for cell proliferation.

With regards to their surface topography, microcarriers are typically categorized by their porosity. In smooth microcarriers, which lack porosity, cells grow on the surface of the carrier as a monolayer. These cell monolayers are also observed in microporous microcarriers, in which the dimensions of the pores do not allow the entry of cells into the internal structure of the microcarriers. However, the small pores allow the penetration of proteins and biochemical signals produced by cultured cells. The secretion of bioactive

molecules and their infiltration into the internal structure of microporous microcarriers thus create a unique biochemical microenvironment within the carriers, which influences the growth and behavior of the cells[83,96,97]. The main disadvantage of both smooth and microporous microcarriers is the low available surface area for cell attachment, which limits cell proliferation. In order to increase the available surface area for cell proliferation, it is possible to produce sponge-like microcarriers that allow the infiltration of cells inside their inner structure, which also protects the cells from mechanical stress[98]. Despite commonly possessing pores with average diameters in the 20–40 μm range, these microcarriers are known as macroporous microcarriers, in order to distinguish them from microporous microcarriers, which do not allow the entry of cells[96,99]. Macroporous microcarriers possess a complex 3D internal architecture consisting of an interconnected pore network, which greatly increases the available surface area for cell attachment, enhancing the cell densities that can be achieved. The porosity, pore dimensions and pore connectivity of these microcarriers can be adjusted to regulate cell proliferation, cell-cell interactions and mass transfer, in order to ensure proper diffusion of nutrients, oxygen, and waste products while also improving the regenerative potential of the cell-laden microcarriers[96,100–102]. Highly porous microcarriers provide a platform with very high surface area/volume ratio, which can be used to develop minimalist tissue engineering approaches[95].

Microcarriers with specific topographical features can also be fabricated in order to present cells with mechanical cues that activate mechanotransduction pathways, modulating cell differentiation, proliferation and interactions between cells. Shape-defined ultrathin microparticles provide a low-material based, quasi-2D platform for cell culture that can be folded to produce a 3D structure through cell-mediated interactions[103].

Recently, disk-like microcarriers with nano-grooved surface patterns have been used as cell carriers with high surface area to induce differentiation of stem cells into an osteogenic lineage[104]. These topodisks were successfully able to promote cell adhesion and control cell orientation while directing cells toward an osteogenic lineage, even without the addition of paracrine signals that promote differentiation. Thus, by adjusting the topographical and biophysical cues displayed by the microcarriers in the core of the microcapsules, it should be possible to tailor the properties of capsule-based systems toward different biomedical applications, through the modulation of cell behavior.

2.3.2. Composition of the microcarriers

A wide variety of biomaterials have been used in the generation of microcarriers, including natural and synthetic polymers, bioactive glass[105] and bioceramics such as hydroxyapatite[106]. Natural polymers used to prepare microcarriers include dextran, gelatin, cellulose and alginate [82,107–110]. Another polymer that has increasingly been used is chitosan, a positively charged polysaccharide obtained through the alkaline hydrolysis of chitin, which can be readily obtained from the exoskeletons of invertebrates such as crustaceans and insects, as well as the cell walls of fungi[111,112]. Polystyrene (PS) is the most commonly used synthetic polymer in commercially available microcarriers[92,113]. Biodegradable synthetic microcarriers have also been thoroughly researched for the fabrication of microcarriers, including polymers such as poly(*L*-lactic acid) (PLLA), poly(glycolic acid) (PGA), PLGA and poly(ε-caprolactone) (PCL)[97,114].

Both PLLA[31,80,81,115,116] and PCL[117–119] microparticles have previously been co-encapsulated with cells within microcapsules. This approach has been successfully used to create platforms that promote osteogenesis[31,116,119]. Due to a lack of cell recognition sites in these polymers, the surface of the microcarriers was coated with collagen to improve cell adhesion. Attachment of cells to microcarriers is dependent on non-covalent interactions between the surface of the microcarriers and proteins on the surface of cells, encompassing electrostatic forces, hydrogen bonds, dipole-dipole interactions and van der Waals forces[120]. Parameters such as the polarity and density of the surface charge, as well as the topography, mechanical properties and wettability of the surface all influence the effectiveness of cell-microcarrier interactions, as reviewed elsewhere[96,121,122]. As such, functionalization of the surface of the microcarriers is often pursued as a strategy to improve cell attachment. This can be accomplished by coating the surface of the microcarriers with bioactive molecules, which often includes components of the ECM, such as laminin, collagen, fibronectin, Matrigel®, vitronectin or small peptide sequences that promote cell recognition and adhesion, most commonly the RGD sequence[123–125]. It is also possible to improve cell attachment by introducing positive charges on the surface of the microcarriers, through functionalization with PLL or small charged chemical groups[92,114,126]. Improving the wettability of the microcarrier surface has also been pursued as a strategy to achieve greater cell adhesion. This can be performed by modifying the surface of microcarriers using methods such as UV-ozone treatment[127]. Surface functionalization is a versatile strategy that can also be used to

produce microcarriers with specialized applications. For example, microcarriers with cell-selective properties can be produced by attaching antibodies to their surface[112]. The immobilization of growth factors on the surface of the microcarriers can also be used to guide cell differentiation. This approach has been employed to produce microcapsules that promote chondrogenesis[115].

3. Biofabrication techniques used in the generation of microcapsules

The development of core-shell capsules with defined characteristics for biomedical applications requires a careful consideration of the methods used in their production. While a wide variety of strategies has been used to produce core-shell structures, including emulsion polymerization[128], and sol-gel methods[48], this section will focus on overviewing more recent biofabrication methods for microcapsule production, including microfluidic platforms, superhydrophobic surfaces, electrospray and layer-by-layer (LbL) assembly, which have been schematized in Figure 2.

3.1. Superhydrophobic surfaces

Superhydrophobic surfaces were first conceptualized by taking inspiration from natural structures such as lotus leaves, which display a remarkable ability to repel water[129]. Superhydrophobic surfaces are produced through the introduction of micro/nano indentations[130] and the modification of surface chemistry[131]. These surface modifications greatly decrease free surface energy, producing surfaces that achieve water contact angles above 150°[131]. These surfaces can then be patterned with wettable regions that permit the formation of individualized droplets, which can be used for a multitude of applications, including the preparation of cell-based structures[132], the development of miniaturized lab-on-a-chip platforms for high-throughput assays[133,134], the preparation of vehicles for the delivery of drugs and bioactive molecules with almost 100% encapsulation efficiency[135] the production of 3D porous scaffolds[136] and the generation of shape-defined hydrogels[137].

Superhydrophobic surfaces can also be used in the development of core-shell multi-layered structures through successive cycles of deposition and crosslinking of polymer solutions. For example, Lima et al. produced multi-layered capsules using methacrylated dextran (DexMA)[42]. In one approach, DexMA was deposited on a superhydrophobic surface,

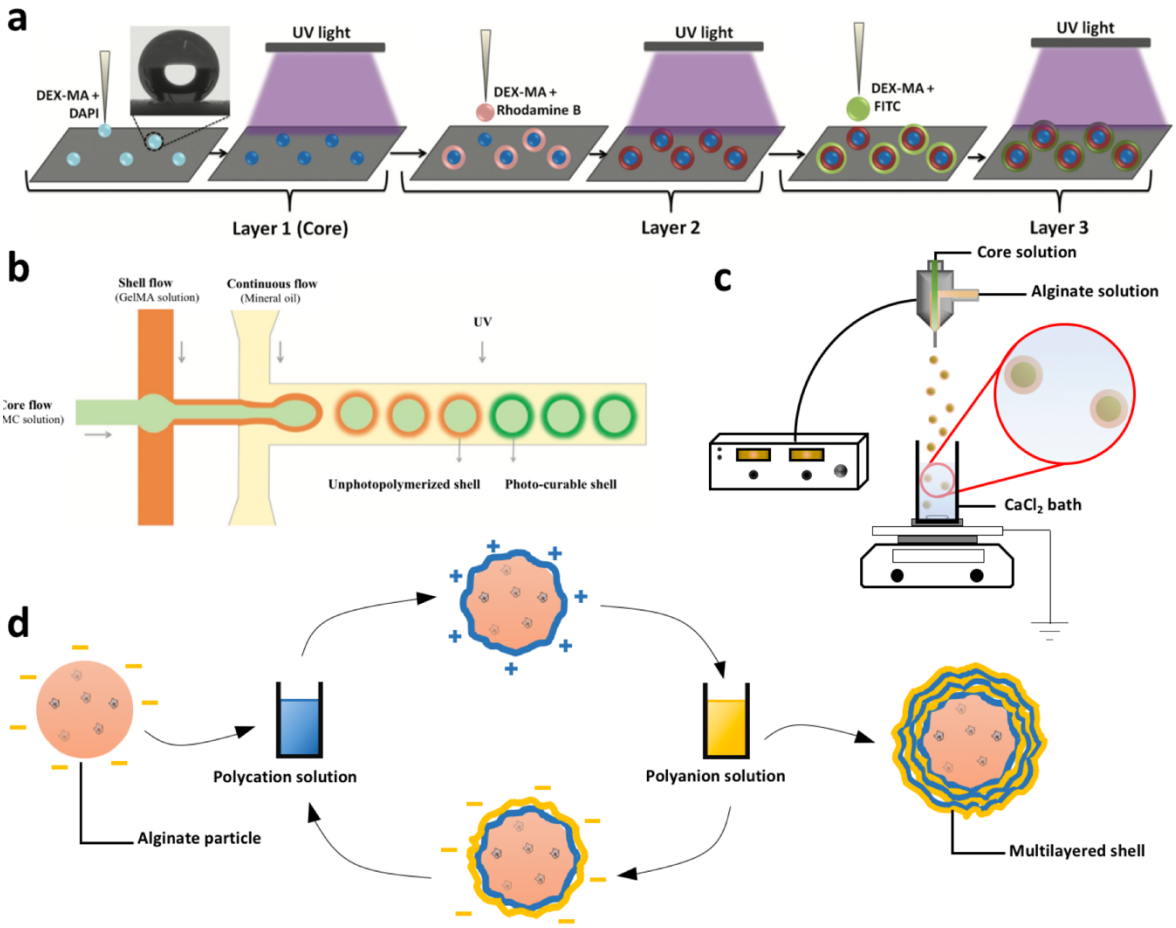

Fig. 2. Schematic representation of biofabrication techniques used in the preparation of core-shell capsules. (a) Superhydrophobic surfaces. Adapted with permission from reference[42]. Copyright 2013 Wiley. (b) Microfluidic devices. Adapted with permission from reference[40]. Copyright 2019 Wiley. (c) Coaxial electrospray. (d) Layer-by-layer assembly.

producing droplets that were crosslinked through UV irradiation to generate microparticles. These initial microparticles were then coated in DexMA solution, which was photopolymerized to produce a shell. By repeating this process, multi-layered shells can be easily produced (Figure 2A). Another possibility that was explored was the initial preparation of DexMA microparticles containing $CaCl_2$. By coating the microparticles with alginate solution, a shell is formed due to the diffusion of Ca^{2+} ions from the core.

The production of liquefied capsules is also made possible due to the reversible gelation mechanism of alginate. By depositing an alginate solution on these surfaces and adding $CaCl_2$ to the resulting droplets, alginate microparticles are produced, which can then be coated with a polymer shell, for example, by depositing a solution of a photopolymerizable material over the microparticles and exposing it to UV light[138] or through layer-by-layer

assembly[118]. The alginate shell can then be liquefied using EDTA, as previously discussed. Additionally, temperature-sensitive materials such as ice and gelatin can also be used to produce liquefied capsules by producing solid microparticles at an adequate temperature, placing the particles on a superhydrophobic surface and coating them in a polymer solution that can be gelified. After gelation, the resulting core-shell structures can be placed at a temperature at which the cores become liquid in order to produce a liquefied system[138]. While superhydrophobic surfaces possess limited usage and low production rates, the shape of their patterns can be adjusted, expanding the possible geometries for microcapsules. This allows the generation of functional units with favourable morphology for the modular assembly of constructs with greater structural complexity[118].

3.2. Microfluidics

Microfluidic platforms are miniaturized devices that can be used to manipulate fluids at a micrometer scale, allowing precise control over the flow of multiple solutions[139]. These platforms have increasingly been used in the fields of drug screening, cell culture and disease modeling, as they allow the imposition of specific conditions, achieving a precise reenactment of specific cell niches, providing accurate simulations of *in vivo* conditions and even allowing the recreation of entire biological systems through organ-on-a-chip and system-on-a-chip platforms[140].

The ability to rigorously adjust flow rates in these platforms can be harnessed to produce highly monodisperse microcapsules, while also offering precise control over the porosity, dimensions, anisotropy and morphology of the capsules[141,142]. The production of microcapsules in microfluidic platforms is based on the flow of a polymer solution, known as the dispersed phase, which intersects the flow of another, immiscible fluid, known as the continuous phase, resulting in the break-up of the dispersed phase, which produces droplets through oil-in-water or water-in-oil emulsions[140,143].

The encapsulation of cells has been accomplished using different configurations, including T-junctions, flow focusing and coaxial flow units[142,144,145]. By combining two droplet forming units, it is possible to generate core-shell microcapsules through double emulsion systems (Figure 2B)[139,141]. By increasing the number of inner flows, or simply by adjusting the solution flow rates, it is possible to produce microparticles with multiple cores, and by increasing the number of droplet forming units, multilayered shells can be obtained[141,143].

A simple procedure to produce core-shell capsules consists of coating the core components with an alginate solution and adding a calcium salt, such as $CaCl_2$, to the continuous phase, producing the shell. This strategy has been employed in the production of capsule-based systems for the formation of spherical embryoid bodies[145]. Alternatively, capsules with alginate shells can also be obtained by mixing insoluble $CaCO_3$ particles into the alginate shell solution, while using a continuous phase containing an acidic solution, which reacts with the $CaCO_3$, releasing Ca^{2+} ions that interact with the alginate resulting in its gelation[146]. The incorporation of crosslinking agents in the continuous phase is also a possible procedure used in the generation of the shell. For example, 4-arm maleimide functionalized PEG (PEG4m) has been used to develop a capsule-based platform through chemical crosslinking[147]. A coaxial flow system was used to envelop an inert PEG core solution in a sheath of PEG4m and break up the two solutions into core-shell emulsions. The outer PEG4m solution is then crosslinked by using an oil phase containing dithiothreitol. By adjusting the core and shell solution flow rates, it is possible to achieve a precise control over the dimensions of both the core and shell, producing a system with highly tunable shell thickness. Polymers functionalized with methacryloyl groups can also be used to produce the outer shell of the droplets, by coupling the microfluidic device with an UV lamp that induces the photopolymerization of the shell.[40,41,148].

The use of microfluidic biofabrication platforms for biomedical applications has been hindered by the need to use organic solvents and oils, which are toxic to cells, denature proteins and are harmful to the environment[149]. A possible alternative is the use of mineral oils, which possess better biocompatibility[150]. Another alternative is the use of aqueous biphasic systems, which are obtained by producing solutions of two incompatible solutes, such as PEG and dextran, at appropriate concentrations[57,151]. These systems can be used to minimize contact between the oil phase and the encapsulated material, while also facilitating removal of the oil. For example, a PEG-diacrylate/dextran system has been harnessed to produce microcapsules for the dual-delivery of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)[152]. In this setup, a continuous phase containing fluorocarbon oil was employed, which was easily washed out without compromising the stability of the encapsulated growth factors. The generated capsules were shown to improve cardiac function after *in vivo* implantation, while also providing a promising platform for the delivery of drugs, signaling molecules and mRNA.

Additionally, aqueous biphasic systems can also be used to develop oil-free biofabrication procedures that eschew the need for organic solvents altogether[153]. A limitation of all-aqueous systems, however, is that it is more difficult to control the properties of the final microcapsules, although this limitation can be circumvented by using an oscillating valve, which can be used to produce microcapsules with specific properties by adjusting oscillation frequencies and solution flow rates[149,153].

3.3. Electrospray

Electrohydrodynamic atomization, also known as electrospray (ES), is a versatile technique used in the generation of both micro and nano scale particulate materials, allowing a high degree of control of the production procedure and generating highly monodisperse particles[154]. The basic principle of ES is the extrusion of a conductive polymer solution through an electrified metal nozzle, which breaks up the liquid, generating droplets that are deposited on a grounded collector[154,155].

The dimensions, morphology and polydispersity of the obtained particles are heavily dependent on operational parameters such as the applied voltage, flow rate, nozzle diameter, tip to collector distance and chosen collector; environmental factors such as temperature and humidity; and the properties of the polymer solution, such as viscosity, surface tension, polymer concentration, solvent conductivity, volatility and permittivity[74,154,156,157]. The effect of processing parameters on the properties of microparticles has been well documented elsewhere[74,158–161]. In summary, higher flow rates will generally increase the electric force required to overcome the surface tension, resulting in an increase in particle diameter, however, they may also lead to an unstable jetting process, decreasing the uniformity of the particles. The applied electric field heavily influences both the jetting mode and the dimensions of the particles. Higher applied voltages will lead to a more thorough break-up of the polymer solution, resulting in smaller particles, while increasing the tip to collector distance will weaken the electrical field, increasing the capsule diameter. Lastly, the selection of an appropriate nozzle is required, as nozzles with lower diameter will produce smaller particles, while higher nozzle diameters may introduce instability in the jetting process. Many different ES configurations are possible, further contributing to the versatility of the technique. Monoaxial setups are able to produce particles in a diverse range of morphologies and shapes[162], including core-shell capsules, which can be obtained through

the incorporation of water-in-oil emulsions[155]. Additionally, liquefied capsules coated in thin membranes can also be obtained using aqueous biphasic systems. Vilabril et al. developed an encapsulation procedure based on the extrusion of a dextran solution containing alginate into a collector bath consisting of a PEG solution containing PLL[163]. Alginate and PLL are two polyelectrolytes of opposing charge, which suffer complexation at the interface of the two phases, leading to the generation of a robust and permeable membrane that envelops a liquid core. These capsules were shown to support the proliferation of MSCs and the formation of cellular aggregates.

Core-shell capsules are also commonly obtained by employing coaxial nozzles (Figure 2C)[61,160]. The formation of capsules with multiple shells is also possible by using a triple coaxial setup[164]. In coaxial ES, the properties of both core and shell solutions will influence the resulting core-shell structures. As such, the surface tension, conductivity, viscosity, permittivity and flow rate of both solutions must be selected appropriately[159,160].

The application of a high voltage electric current during microcapsule generation could hinder the survival of cells, however, it has been shown that cell proliferation is not affected by the electrospraying process when the strength of the applied electrical field is below 3 V/cm[165]. When compared to microfluidics, coaxial electrospray presents numerous advantages, as it is a one-step process that requires a single solvent, eschewing the need for the use of organic solvents or complex solvent systems. This also facilitates the recovery of the microcapsules after production and reduces the amount of waste produced. Furthermore, ES provides greater control over the properties of the microcapsules, as it relies on a greater amount of parameters that can be modulated and optimized[154]. As the implementation of microcapsule based strategies advances to the clinical stage, coaxial ES could provide an avenue toward the large scale production of microcapsules, as it can be performed in sterile conditions, it is easy to use, it employs higher flow rates and it can achieve high production and encapsulation rates, with further potential for application at industrial scales by employing multiple nozzles[71,166–168].

3.4. Layer-by-layer assembly

LbL assembly has been explored as a simple, versatile, low-cost and environmentally safe approach for the generation of ultrathin films using a vast range of starting materials, including enzymes, polymers, ceramics, metals and even cells[169,170]. As a cell encapsulation

method, it can be used to generate microcapsules enveloped in thin multilayered membranes with tunable structure, permeability and composition[171,172]. Despite being a time-consuming process with reduced scalability, LbL assembly can be performed in mild conditions while using aqueous solvents, which is advantageous for the generation of suitable platforms for TERM applications. The encapsulation of pancreatic islets through LbL assembly was a pioneering approach in cell encapsulation[33].

Core-shell systems can be obtained through the sequential adsorption of polymers on the surface of a sacrificial core, which acts as a template. The assembly of the multilayered shell can rely on multiple forces acting between the chosen materials, including hydrogen bonds, hydrophobic interactions, covalent bonds and electrostatic interactions[173,174]. The generation of microcapsules for cell encapsulation and delivery of bioactive molecules has often relied on the electrostatic forces between polyelectrolytes with opposing charges. Appropriate polycations and polyanions are selected and sequentially deposited to produce a polymer shell. In this process, the template cores are coated in a dilute solution of polyelectrolyte in order to produce the first layer of the membrane. The capsules are then washed, and placed in a solution of a second polyelectrolyte, with opposing charge. This process is repeated until the shell has reached the desired thickness (Figure 2D)[175]. The inner core of the capsules can then be liquefied, dissolved or eliminated, allowing the generation of microcapsules with solid, liquefied or hollow cores[66,173,176].

Alginate–poly-*L*-lysine–alginate (APA) capsules are one of the most prominently used systems in cell encapsulation. These capsules contain an alginate core surrounded by a multilayered shell of poly-*L*-lysine (PLL) and alginate. Cells are mixed in an alginate solution, which is used to create solid microparticles through ionic crosslinking in a $CaCl_2$ solution, often through ES[177]. The microparticles are then coated with a first layer of PLL and a second layer of alginate, through LbL. Further layers can be added, and it is also possible to liquefy the alginate core. These systems have been used to deliver stem cells[32,178], pancreatic islets[179], hepatocytes[180] and Chinese hamster ovary (CHO) cells[181]. While PLL is commonly selected as a polycation in LbL assembly, it has been shown to be toxic to cells at higher concentrations and if it is not properly bound to the capsules, it can potentially be immunogenic, resulting in fibrosis[182,183]. As such, other polycations have been researched as possible alternatives, such as chitosan[184], poly-*L*-ornithine (PLO)[185], poly(allylamine)[186] or

copolymers of PLL and PEG[187], which have allowed the production of capsules with reduced immunogenicity, enhanced biocompatibility and improved mechanical properties.

4. Biomedical applications of core-shell microcapsules

In recent decades, core-shell capsule-based platforms have been prominently explored in different fields of medicine, tissue engineering and cell culture with the purpose of designing effective encapsulation systems for the delivery of cells, tissues, drugs and proteins. Possible applications have included the delivery of pancreatic islets to individuals suffering from type I diabetes, as well as the development of TERM approaches to bone and heart disorders. They have also been suggested as bioinspired scaffolds for 3D cell culture and disease modeling. In this section, these contributions will be explored.

4.1. Type I Diabetes

Research efforts into the potential biomedical application of microcapsules were pioneered by Lim and Sun, in their attempts to develop therapeutic approaches to type I diabetes mellitus, a metabolic disorder caused by an autoimmune response to β-cells, located in pancreatic islets[33,191]. The transplantation of pancreatic islets has been proposed as a therapeutic strategy to restore β-cells in the pancreas and reduce the need for insulin injections, however, this process has been hindered by rejection of the transplanted islets[191]. Initially, it was shown that encapsulation in microcapsules prolonged the survivability of transplanted islets in rats from 8 days to 3 weeks[33].

The results obtained by Lim and Sun were later confirmed by O'Shea et al.[179], who also extended the survival period of islets to a full year, in rats, through encapsulation in APA microcapsules. Early clinical studies regarding the transplantation of encapsulated islets in a human patient showed that this approach was capable of granting insulin independence for a period of 9 months[34]. As previously mentioned, however, PLL presents several limitations,

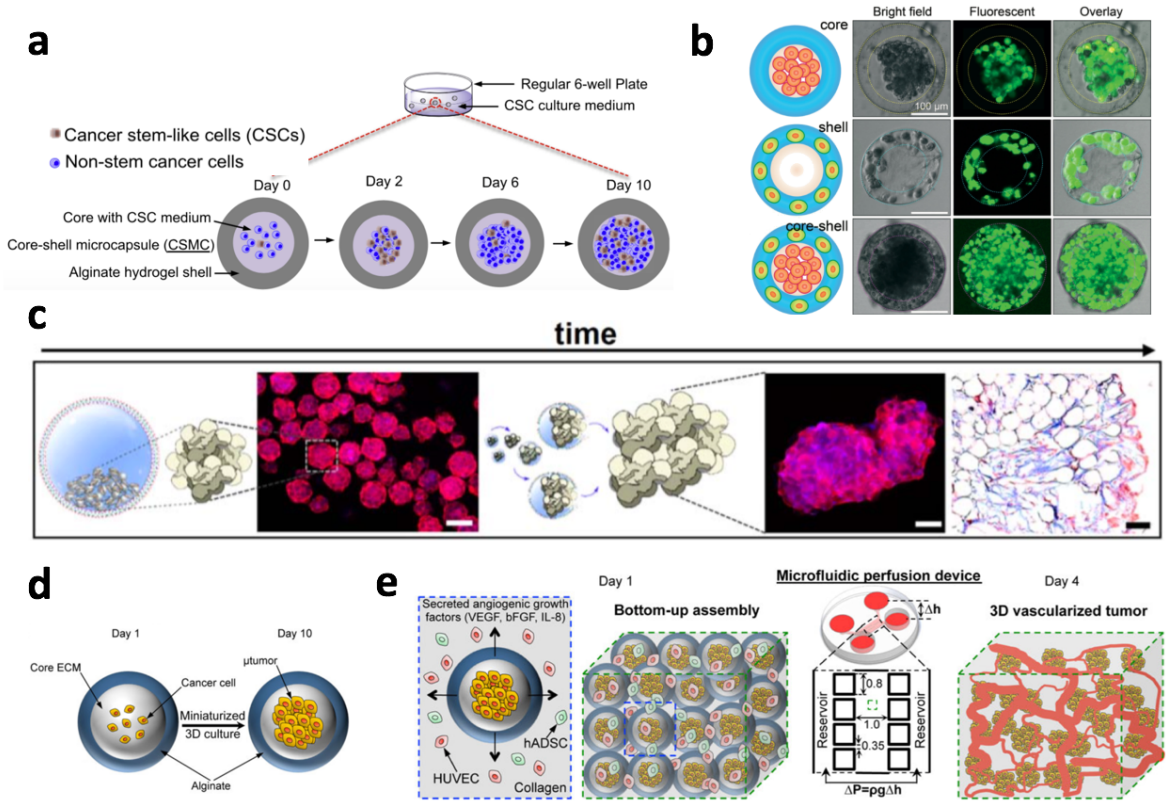

Fig. 3. Outline of biomedical applications of microcapsules. (a) 3D platforms for the culture, proliferation and differentiation of cells, with potential for the enrichment of rare cell subpopulations. Adapted with permission from reference[188]. Copyright 2014, Elsevier. (b) Development of compartmentalized platforms for a more accurate recreation of *in vivo* tissue architecture[189]. (c) Assembly of complex structures, such as microtissues and spheroids through the self-assembly and aggregation of cells within the capsules. This can be aided by seeding cells alongside surfaces that permit cell attachment, such as microparticles. Scale bars represent 50 μm. Adapted with permission from reference[119]. Copyright 2019, IOP Publishing (d) Development of robust disease models to evaluate the effects of mechanical cues on cell behavior and the efficacy of drug candidates. (e) Production of functional units for the modular assembly of larger constructs, and even vascularized structures. Adapted with permission from reference[190]. Copyright 2017, American Chemical Society.

which has prompted its replacement with other polymers, such as chitosan[192] or PLO[193]. Wang et al.[194] explored over one thousand polyelectrolyte combinations and produced multicomponent capsules with highly tunable dimensions and mechanical properties using sodium alginate, cellulose sulphate, poly-methylate-co-guanidine, calcium chloride and sodium chloride.

The co-encapsulation of pancreatic islets with other cell types, such as mesenchymal stem cells (MSCs)[195,196] or Sertoli's cells[185], is a strategy that has been shown to extend islet survival, while also improving the regenerative potential of the microcapsules. Recently, it

has also been shown that inclusion of hyaluronic acid in the matrix of the alginate core enhances the survival of insulin-producing cells[197].

Core-shell microcapsules also provide a viable approach to visualize transplanted islets, through the co-encapsulation of islets with tracking agents that allow non-invasive imaging of the cells. To minimize the toxicity of these compounds, capsule-in-capsule systems have been developed, which provide a highly compartmentalized platform that functions as a vehicle for islet transplantation, while also facilitating the subsequent monitoring of islet location and survival[198].

Table 1. Summary of core-shell microcapsule platforms developed for the treatment of type I Diabetes. This summary covers the materials used to produce both the core and shell of the capsule, the technique used to generate the capsules and the biological materials encapsulated in each structure.

Shell material	Core structure	Production technique	Encapsulated material	Ref.
PEI/PLL	Alginate	LbL	Pancreatic islets	33
Alginate/PLL	Alginate	LbL	Pancreatic islets	179
Alginate/PLL	Alginate-HA	Ionotropic gelation, LbL	Rat Ins1E cells	197
Alginate/PLO	Alginate	LbL	Pancreatic islets, Sertoli's cells	185
Alginate/PLO	Alginate	Ionotropic gelation, LbL	Pancreatic islets (core) FGF-1 (Alginate shell)	193, 202, 205
PLL	Pancreatic dECM	Ionotropic and thermal gelation	Insulin producing cells derived from adult human liver cells or MSCs	64
Alginate/PLL	Alginate	Ionotropic gelation, LbL	Adult porcine islets	199

One of the major obstacles to the long-term viability of encapsulated islets is the development of hypoxic conditions in the capsules, due to the high oxygen demand of pancreatic islets.[193,199]. The inclusion of oxygen carrier materials has been explored as a strategy to reduce damage caused by hypoxia in the short-term[200]. A review of these materials has been provided elsewhere[201]. A long-term approach would be the promotion of vascularization at the site of implantation, in order to increase blood flow and oxygen supply in the affected area. This can be accomplished through the delivery of pro-angiogenic factors such as fibroblast growth factor 1 (FGF)[202], PDGF[203] and VEGF[204]. A possible application of this strategy was studied by Opara and coworkers, who attempted the co-delivery of cells

and pro-angiogenic growth factors in a compartmentalized platform. Pancreatic islets were encapsulated in the core of an alginate-PLO-alginate microcapsule and FGF-1 was incorporated in the shell[193,202,205]. Long-term viability of the capsules was achieved, particularly when microcapsules were delivered to the omentum, a highly vascularized tissue, highlighting the importance of proper vascularization in islet survival[202].

4.2. Bone defects

The recovery of bone defects and bone fractures is typically promoted through the replacement of damaged bone using a surgical procedure known as bone grafting. Currently, bone grafting is the second most common tissue transplantation procedure, after blood transfusion[206]. However, this procedure holds many limitations. Autologous bone grafts require two surgical procedures, may damage the donor site and yield a limited amount of tissue. Allogeneic bone grafts, on the other hand, display inferior healing capabilities, while also carrying the risk of immune rejection and of transmitting pathogens from donor to patient[207–209]. The implantation of metals, ceramics or biomaterial scaffolds has also been explored, however, these materials often display inadequate mechanical properties, poor integration with the native tissue or reduced cell attachment. Applying TERM approaches to the treatment of bone defects could provide new options for enhanced graft incorporation, formation of bone tissue and development of engineered bone constructs[210]. Core-shell structures are optimal for the delivery of both cells and proteins in bone TE strategies, as they can better mimic the hierarchical structure of bone when compared to other microencapsulation platforms[211].

Multilayered hollow-core microcapsules for the delivery of osteogenic growth factors were designed by Facca et al. using LbL[176]. Bone morphogenic protein 2 (BMP-2) and transforming growth factor β1 (TGF-β1) were incorporated in a PLL/poly-*L*-glutamic acid (PLL-PGlA) multilayered shell. The capsules were shown to increase the stability of the growth factors and induce the formation of bone *in vitro*, in the presence of ESCs. The hollow core microcapsules were then embedded in an alginate gel accompanied by ESC-derived embryoid bodies. The resulting gel was shown to induce bone formation and vascularization after *in vivo* implantation. Microtissues with enhanced mechanical properties were produced by Luo and coworkers by combining an open porous gelatin shell with a core generated from demineralized bone matrix loaded with BMP-2[5]. The core-shell structures

exhibited high cell seeding efficiency, sustained release of BMP-2, higher viability of seeded bone marrow mesenchymal stem cells, and enhanced calcium deposition and mineralization when compared to gelatin derived microtissues. When implanted *in vivo*, the core-shell microtissues were shown to promote the formation of bone.

Table 2. Summary of core-shell microcapsule platforms for application in bone TE. This summary covers the materials used to produce both the core and shell of the capsule, the technique used to generate the capsules and the biological materials encapsulated in each structure.

Shell material	Core structure	Production technique	Encapsulated material	Ref.
PLL/PGlA	Hollow core	LbL (sacrificial PS template)	BMP-2, TGF-β1 (incorporated in shell)	[176]
Calcium silicate	Alginate	Ionotropic gelation	Proteins	[49]
PLL/Alginate/Chitosan	Alginate	LbL	ASCs, endothelial cells, PLLA microparticles	[31,116]
Gelatin	Demineralized bone matrix	Micro-stencil array chip	BMP-2 (core) BMSCs (shell)	[5]
PLL/Alginate/Chitosan	Alginate	Electrospray, LbL	ASCs, osteoblasts, PCL microparticles	[119]

Liquefied core microcapsules have also been proposed as a platform for the fabrication of bone tissue for TE applications. For example, SaOs-2 cells encapsulated in liquefied alginate core microcapsules coated with a multilayered membrane of chitosan and alginate retained viability after encapsulation[66]. Further studies showed that co-encapsulation of adipose-derived stem cells (ASCs) and endothelial cells in liquefied microcapsules can induce osteogenic differentiation of the ASCs even in the absence of osteogenic growth factors, providing an effective strategy for the development of bone tissue[31,116]. Furthermore, the differentiated ASCs were shown to produce and secrete paracrine factors such as BMP-2 and VEGF, which travel from within the capsules to the surrounding environment through diffusion, thus revealing the possibility of using the encapsulated cells as biofactories for the production and sustained release of biochemical signaling molecules. When these capsules were implanted *in vivo*[116], the formation of mineralized tissue was observed. Moreover, a bone-like tissue could be observed even in capsules that were not subjected to *in vitro* pre-differentiation procedures, indicating that the microcapsules could be readily implanted after preparation[31,116]. Liquefied microcapsule platforms have also been used to develop bone

microtissues through the co-encapsulation of osteoblasts, adipose-derived stem cells and PCL microparticles (Figure 3c)[119]. In dynamic culture conditions, the hydrodynamic shear improved cell-cell interactions, generated larger cell aggregates and induced the osteogenic differentiation of ASCs even in the absence of osteogenic growth factors and osteoblasts. Furthermore, microcapsules co-encapsulating ASCs and osteoblasts in a dynamic environment displayed clear signs of mineralization, such as the growth of apatite-like minerals, similar to those found in native bone tissue[116].

4.3. Cardiovascular diseases

Despite the development of numerous therapeutic and preventive measures to address cardiovascular disease in the last few decades, these disorders remain the leading cause of death worldwide, accounting for 17.8 million deaths in 2017 and for an expected 22.2 million deaths by 2030[212,213]. As such, there is an urgent demand for novel approaches to produce cardiac cells, regenerate heart tissue and study cardiac pathophysiology. Core-shell microcapsules provide a versatile platform that can be directed toward these applications. For example, the use of coaxial ES to encapsulate ESCs in liquid core alginate microcapsules has been pursued as a scalable, biomimetic, cost-effective and highly tunable approach to produce cardiomyocytes for cardiac transplantation[71,150,214]. After 7 days, ESCs formed cellular aggregates with dimensions comparable to those of previously used methods, while requiring a much lower number of initial cells[71]. Furthermore, this approach allowed cells to maintain a greater degree of pluripotency than previous approaches while potentially allowing the single step production of millions of capsules per day, providing a sustainable source of embryoid bodies for tissue regeneration. Differentiation of ESCs into the cardiac cell line can be achieved by applying bone morphogenic protein 4 (BMP-4) and FGF-2, producing beating aggregates that closely mimic the cellular composition of native cardiac tissue. In a later work, Zhao et al. transplanted the obtained aggregates into the infarcted heart, improving heart function[65]. The aggregates were released from the core-shell microparticles and re-encapsulated without a noticeable influence on cell viability or the integrity of the aggregates.

Promoting vascularization has also been suggested as a potential therapeutic approach in the treatment of ischemic heart disease, in order to ensure the oxygenation of transplanted cells and tissues. Zhang et al. performed the genetic modification of CHO cells to produce and

secrete VEGF, in an attempt to improve vascularization. The cells were delivered to infarcted tissue, in rats, using liquid core APA microcapsules[181]. It was shown that the microcapsules were stable post-implantation and displayed lower immunogenicity when compared to non-encapsulated cells, indicating that the capsules were successfully isolating cells from the immune system. Furthermore, the continuous release of VEGF stimulated angiogenesis and restored cardiac function. The co-encapsulation of MSCs and Schwann cells in liquid core APA microcapsules has also been shown to induce angiogenesis[215]. MSCs secrete a wide variety of growth factors and chemical signals that promote vascularization and the Schwann cells extend the viability of MSCs, while also yielding increased density of newborn capillaries in treated areas.

Table 3. Summary of core-shell microcapsule platforms used in myocardial TE. This summary covers the materials used to produce both the core and shell of the capsule, the technique used to generate the capsules and the biological materials encapsulated in each structure.

Shell material	Core structure	Production technique	Encapsulated material	Ref.
Alginate/PLL	Alginate	Electrospray, LbL	CHO cells	181
Alginate/PLL	Alginate	Electrospray, LbL	MSCs, Schwann cells	215
PEGDA	Dextran	Microfluidics	VEGF, PDGF	152
PLL	Alginate	Ionotropic gelation	ESCs	214
Alginate	Sodium carboxymethyl cellulose solution	Microfluidics	ESCs	150
Alginate	Sodium carboxymethyl cellulose solution	Coaxial electrospray	ESCs	65, 71
Silica	GelMA	Microfluidics, sol-gel	Cardiac progenitor cells	48

4.4. 3D cell culture

In vitro cell culture has typically relied on 2D plastic substrates, which cannot fully recreate the microenvironment and cell interactions present within native tissues. This has become a widely recognized limitation of *in vitro* models used in the study of diseases and drug screening[216]. Biomimetic 3D platforms can emulate the architecture and properties of living tissues more accurately, providing efficient platforms to model cell behavior. By altering the composition of the core, it is possible to tailor the stiffness of the materials encapsulated alongside the cells, providing a medium to evaluate the effects of mechanical cues on cell behavior[190]. As previously mentioned, liquid core microcapsules present numerous benefits

as *in vitro* cell culture platforms when compared to solid core structures, as they facilitate cell-cell interactions and the self-assembly of cells into more complex structures while preserving cell viability[61].

Core-shell microcapsules thus supply an opportunity to improve cell culture methods, by tailoring their inner microenvironment toward different cell types, which can be accomplished owing to the high tunability and versatility of available production methods. Moreover, it is possible to design microcapsules that allow the compartmentalized encapsulation of different cells[217]. For example, Chen and coworkers produced a "liver in a drop" by designing an encapsulation system in which hepatocytes are incorporated in the liquid core of the capsule while fibroblasts were embedded in the alginate shell (Figure 3b)[189]. This structure keeps the two cell types separated, in order to preserve their specific functions, while still allowing adequate cell-cell interactions to occur, resulting in a promising *in vitro* model for liver function.

While the structure of the microcapsules can be modulated to adjust interactions between encapsulated cells, it is also possible to construct systems that promote interactions with cells outside the capsules. For example, Correia et al. co-encapsulated osteoblastic cells and PCL microparticles in liquefied core microcapsules coated in a multilayered membrane of alginate, PLL and chitosan and enveloped in an outer layer of RGD-functionalized alginate[117]. When transferred to a 2D cell bed of fibroblasts and human umbilical vein endothelial cells (HUVECs), the RGD domains in the outer surface of the microcapsules promoted cell attachment to the outer surface of the membrane, and allowed the aggregation of cell-coated microcapsules, producing constructs with complex hierarchical structures. In a follow-up work, it was shown that adjusting the composition of the outer layer of the microcapsule shell can promote the modulation of surrounding macrophages toward a pro-regenerative behavior, an effect which is enhanced through the encapsulation of cells that can communicate with the macrophages through paracrine signaling[218]. This demonstrates that the properties of microcapsules can be tailored in order to guide native cells toward desired phenotypes.

These 3D cell culture systems hold great promise in the modeling of diseases for high-throughput assays. The design of robust tumor models for drug screening is an ongoing challenge in oncogenic research[219,220]. Core-shell microcapsules have been explored as suitable platforms for the formation of cell spheroids[221], which can represent accurate models

for cell-cell interactions and diffusion of nutrients and drugs in tumors[222]. The possibility of encapsulating cells in separate compartments enables an effective recreation of the tumor microenvironment[219,223]. Furthermore, microcapsules can provide physicochemical and mechanical cues that can direct gene expression and cell function in order to study different aspects of tumor growth[224,225]. Alessandri et al., for example, designed an elastic capsule-based platform that can be used to evaluate the force exerted by expanding multicellular spheroids, simulating the pressure exerted by growing tumors on surrounding tissues, while also providing insight into the influence of mechanical cues on tumor progression[225]. This system was even able to induce the formation of spheroids in recalcitrant cell lines.

Table 4. Core-shell microcapsule platforms employed in tumor models. This summary covers the materials used to produce the capsules, the technique used in their generation and the encapsulated cells.

Shell material	Core structure	Production technique	Encapsulated material	Ref.
Alginate	Sorbitol solution	Microfluidics	CT26, HeLa and S180 cells	[225]
Alginate/PLL	Alginate	Electrospray/LbL	HT-29 cells	[224]
Alginate	Sodium carboxymethyl cellulose solution	Coaxial electrospray	Prostate CSCs	[188]
Alginate	Alginate	Microfluidics	MCF-7 cells	[221]
Alginate	Collagen + alginate + Matrigel®	Microfluidics	MCF-7 cells	[146]
Alginate	Collagen I, Collagen I + Alginate	Microfluidics	MCF-7 cells	[190]
Alginate	Cell culture medium	Microfluidics	MCF-7 cells (core) Human Mammary Fibroblasts (shell)	[219]

Bioencapsulation in liquefied core capsules has been proposed as a timely and cost-effective procedure to enrich cancer stem-like cells (CSCs), a rare subpopulation of cells highly involved in the initiation, expansion, metastasis and resistance of tumors (Figure 3a)[188]. Core-shell microcapsules have also been successfully used as modular units in the bottom-up assembly of vascularized constructs(Figure 3d,e)[190]. Cancer cells were suspended in a collagen I solution and encapsulated in alginate microcapsules, generating avascular microtumors. To produce a vascularized structure, the microcapsules were incorporated in a

collagen I gel alongside ASCs and HUVECs, resulting in a 3D capillary network surrounding the microtumors. When compared to 2D-cultured cells, the vascularized construct was shown to be more tumorigenic and more resistant to anti-cancer drugs, providing a robust tumor model for drug testing.

In summary, core-shell capsules represent an important breakthrough in the development of 3D culture systems for the study of diseases and potential treatment options. Future developments in this field will require the development of capsule-based systems that can better emulate human tissues, which will require a careful selection of appropriate core and shell components. One possibility under consideration is the incorporation of human derived hydrogels, which would minimize the use of xenogeneic materials, thus creating structures that are more faithful to the composition of human ECM[223,226].

5. Conclusions and future directions

A wide variety of materials and techniques have been harnessed to produce core-shell microcapsules, generating versatile platforms that can be precisely fine-tuned in terms of their composition, mechanical properties and 3D structure, in order to recreate different physiological niches. These systems have already been implemented in tumor modeling, bone, pancreatic and heart tissue engineering, as well as lung[227], cartilage[115] and hepatic[147,189] tissue engineering. At present, the application of core-shell microcapsules in a clinical setting still presents significant challenges, due to factors such as a lack of appropriate vascularization of the constructs *in vivo*, reduced scalability of production techniques and insufficient long-term stability of encapsulated materials. Further research into these platforms will continue to tackle these issues. Recent studies have also focused on the development of long-term strategies for the storage and preservation of encapsulated structures, a requirement for wide application of these platforms at a clinical level[67].

While originally conceived as a platform for cell immunoisolation, recent works on microcapsules have also attempted to move away from this framework, shifting the focus toward cooperation with immune cells, by guiding them toward a pro-regenerative phenotype[228]. Through careful selection of the biomaterials and cells used to produce these microcapsule-based systems, it has been shown that they can be used as immunomodulatory platforms for tissue regeneration[218].

As bioencapsulation technologies advance, it is envisioned that these platforms will become a vital tool in biomedical research, with possible applications at all stages of the value chain, from the design of effective disease models for pre-clinical studies, to the generation of large amounts of cells and tissues for clinical application, to the development of novel systems for cell transplantation, delivery of drugs and release of paracrine factors.

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Conflict of Interest

The authors declare no conflict of interest.

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