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Biodegradable liposome-encapsulated hydrogels for biomedical applications: a marriage of convenience

Santiago Grijalvo,[a,b] Judith Mayr,[c] Ramon Eritja[a,b] and David Díaz Díaz*[a,c]

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Hydrogels are hydrophilic three-dimensional networks with demonstrated potential for medical and pharmaceutical applications. Specifically, biopolymer-based hydrogels offer certain advantages over synthetic polymers in terms of biocompatibility and biodegradability. Because of their inherent properties, hydrogels are able to efficiently encapsulate and liberate in a controlled release manner, different hydrophobic and hydrophilic therapeutic molecules, including nucleic acids, proteins and antibodies. Several strategies have been reported in the literature to minimize the potential burst release of encapsulated drugs, thus preventing their local accumulation and consequent toxic responses. Within this context, liposomes embedded in hydrogels have emerged as an attractive strategy to reduce this undesirable effect. This tutorial review covers a selection of the most promising cationic, neutral and anionic biopolymer-based hydrogels containing liposomes, niosomes or vesicles for drug delivery or tissue engineering applications.

[a]*Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain*
[b]*Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER BBN), Spain*
[c]*Institute of Organic Chemistry, University of Regensburg, Universitätstrasse. 31, D-93040 Regensburg, Germany. E-mail: David.Diaz@chemie.uni-regensburg.de; Fax: +49 941 943-4121; Tel: +49 941 943-4373*

1. Introduction

Over the past few decades, synthetic progress towards fabrication of new polymeric materials that respond to external stimuli (*e.g.*, temperature, light, pH)[1–4] has stimulated the development of novel approaches for drug delivery,[5,6] tissue

Santiago Grijalvo

Santiago Grijalvo was born and grew up in Madrid. In 2000, he received his Bachelor's degree in Chemistry (with an emphasis in Organic Chemistry) from the Autonomous University of Madrid. After finishing his studies, he was hired as an intern at GlaxoSmithKline for one year. In 2002 he moved to Barcelona to join Dr Antonio Delgado's research group to do his PhD studies focusing on the synthesis of modified sphingolipids combinatorial libraries at the Institute of Research Chemistry (IIQAB-CSIC). In 2007 he joined Prof. Dr Ramon Eritja's research group as a post-doctoral researcher at the Institute of Advanced Chemistry (IQAC). Since then, he has participated in several research projects focusing on improving the cellular uptake of nucleic acids using either covalent or electrostatic strategies.

Judith Mayr

Judith Mayr studied Chemistry at the University of Regensburg, receiving her Bachelor's degree in 2011 and her Master degree in 2013, respectively. Currently she is doing her PhD in the group of Prof. Díaz Díaz. Her main research interest lies in the use of soft material for the formulation and delivery of therapeutically active molecules.

engineering[7] and nanobiotechnology.[8,9] This environmental responsiveness triggers major changes in both the physicochemical and self-assembling properties of such macromolecular systems, which can be used to favour the encapsulation/release of active molecules. Indeed, there are numerous examples in the literature where synthetic polymers have been used either as therapeutic macromolecules or as drug delivery vehicles upon combination with small drugs.[10–12]

However, for real applications, synthetic polymers must fulfill a series of requirements such as being non-toxic, non-immunogenic and water-soluble together with safe excretion properties, which in most cases become very difficult challenges. It is at this point where biocompatible and biodegradable natural polymers represent a versatile and renewable alternative to synthetic polymers. In general, biopolymer-based materials are mainly made of polysaccharides and are usually key components in food industrial formulations acting as emulsifying, gelling and/or stabilizing agents.[13] Furthermore, they have also been proven to be promising materials for biomedical applications including drug delivery and regenerative medicine.[14–17]

1.1. Hydrogels

Polymeric hydrogels are hydrophilic 3D polymer networks capable of absorbing large amounts of water in a similar way to body tissues. This property allows hydrogels to encapsulate therapeutic molecules and protect them from rapid degradation,[18–20] making them useful materials for pharmaceutical and medical purposes.[18,19]

In 1960, Wichterle and Lim[21] reported the use of poly-2-hydroxymethacrylate (PHEMA)-based hydrogels for contact lens applications. Since then, the extensive research carried out in the field of hydrogels has allowed the development of not only polymeric networks based on synthetic polymers such as poly(vinyl alcohol),[22,23] poly(ethyleneimine)[24] and poly(ethylene oxide),[25] among others, but also based on natural polymers such as alginate[26] and chitosan.[27]

Another important feature of many polymeric hydrogels is their ability to release entrapped therapeutic molecules in a well-controlled manner. This property is usually governed by passive diffusion mechanisms and can also depend on additional factors (*e.g.*, cross-linking degrees, hydrogel mesh sizes, stimuli-sensitive hydrogel capacity, *etc.*). One major aspect to take into account during release experiments is the possibility of obtaining undesired initial liberation of the drug immediately upon contact with the release medium. Although this undesirable effect could be positive in exceptional therapeutic strategies, it usually causes an unexpected *in vivo* toxicity due to the local presence of large amounts of drug ("dose-dumping"). Such a process is known as burst release effect.[28]

Although the exact mechanism and prediction of burst release in hydrogel/drug systems has not been elucidated yet,[29,30] several strategies have been reported to minimize this effect. These include, for example, increasing the cross-linking density of the material, coating additional drug-free layers or using drug surface extractions prior to *in vivo* usages.[31–33]

1.2 Hydrogel classification and basic structure

Hydrogels are classified according to several characteristics. For example, they can be classified on the basis of ionic charges (neutral, anionic, cationic or ampholytic hydrogels), the nature of side groups (*e.g.*, neutral or ionic), their physical structure (*e.g.* amorphous, semi-crystalline, hydrogen-bonded

Ramon Eritja

Ramon Eritja studied Chemistry and Pharmacy at the University of Barcelona, receiving his Ph.D. from the University of Barcelona in 1983. He did postdoctoral work in Dr Itakura's and Caruthers's groups. In 1990, he became group leader at CSIC and then at EMBL. He returned in 1999 to IQAC-CSIC and in 2012 he was appointed director of the institute (IQAC). He is a member of the CIBER-BBN network since 2006. His research focuses on oligonucleotide synthesis for biomedical and nanotechnological applications.

David Díaz Díaz

David Díaz Díaz (born in 1974, Tenerife) received his PhD in Chemistry at the University of La Laguna. In 2002, he joined Prof. Finn's group as postdoc at The Scripps Research Institute (San Diego, USA). Since 2006, he has held various positions in academia and industry ('Ramón y Cajal' Researcher, Autonomous University of Madrid, Spain, 2006; Sr. Chemist, The Dow Chemical Company, Switzerland, 2007; Tenured Scientist, CSIC, Spain, 2009; Alexander von Humboldt Experienced Researcher, University of Regensburg, Germany, 2010). In 2013, he was named recipient of the DFG Heisenberg Professorship. He has received the Young Investigator Award from the Polymer Network Group (Japan, 2014) and he is Editor-in-Chief of Gels. His main research interest focuses on the development of advanced functional soft materials.

structures, super-molecular structures and hydrocolloidal aggregates), the crosslink nature (*e.g.* chemical or physical) and their preparation method (*e.g.*, homo- or co-polymers).[34]

Hydrogels can also have many different physical forms and have been used in varying therapeutic applications. Some examples include solid molded forms (*e.g.* contact lenses), pressed powder matrices (*e.g.* pills and capsules), beads (*e.g.*, drug delivery), microparticles (*e.g.* wound treatments), coatings (*e.g.* implants or catheters) and membranes or sheets (*e.g.*, a reservoir in a transdermal delivery patch).

An important issue to consider for drug delivery applications is to understand the transport mechanisms of the drug through the hydrogel matrix, which is usually governed by several parameters such as polymer composition, porosity, degradation rate, size and gel swelling.[35] The mesh size is an important property that is related with degradability, mechanical strength and drug diffusion. Thus, as the gel swells, its mesh size increases and thus facilitates the corresponding drug release in solution. As a consequence, a good number of factors has to be considered and this requires a high degree control over some properties when designing hydrogels. Hence, hydrogel crosslinking degree, stimuli (pH, temperature) or monomer chemical structures are important parameters to design appropriate diffusion patterns of model drugs.[36] Most hydrogels have the possibility of tuning their mesh sizes with varying dimensions and patterns that can range from micrometers to nanometers (5 to 100 nm) approximately in their swollen state. Specifically, microgels have been designed as novel platforms for both local sustained drug release and their ability to mimic the native tissue mechanical properties (3D-cell culture environment) as well as a platform for local protein release. This application has allowed both a rational design and the development of microfabrication strategies that have helped to provide new insights into regeneration and tissue repair applications.[37]

Nanogels have also become promising materials to be mostly used in systemic drug delivery applications. Moreover, they have shown certain advantages over other existing drug delivery systems. Therefore, a decrease in the hydrogel size may produce a faster response for gel swelling and thus increasing the material response and, consequently, the anticipated drug action.[38] The recent advances of click chemistries affording the effective introduction of targeting ligands combined with the use of either polysaccharide-based crosslinked agents or cross-linkers containing biodegradable linkages have allowed the preparation of functional nanogels with narrow size distributions that have facilitated both cellular uptake and have avoided undesirable aggregation processes.[39,40] Such control of microgel and nanogel properties has allowed extensive research in the development of synthetic strategies for the preparation of such materials. For example, a variety of processes like photolithographic, microfluidic techniques, micromolding methods as well as the use of heterogeneous solution, aqueous homogeneous gelation, spray drying methods, chemical crosslinking of dextranes or heterogeneous radical polymerizations are the most representative methods that have been described and revised in the literature.[41]

1.3. Liposomes and preparation methods

Liposomes (self-assembled lipid vesicles) are one of the most used nanosystems to encapsulate drug molecules. Because of their nanometer size, these kinds of non-viral carriers have become one of the most studied drug delivery systems for clinical applications to date.[42–44] Since their beginning in the 60s, the field of liposomes has undergone a major change with respect to the development of novel responsive vesicles with at least one lipid bilayer[45,46] and their applications in hyperthermia processes for cancer treatment.[47,48]

A good number of encapsulation strategies where liposomes are used have been reported to date. For instance, an elegant and recent approach consists of introducing different types of nanoparticles (*e.g.*, gold and silver nanoparticles, SPIOs or lipid vesicles) and obtaining the corresponding "liposome–nanoparticle" hybrids using liposomes as templates.[49,50] The methodology involved in the synthesis of these constructs has helped us to obtain systems that have enhanced their biocompatibility and colloidal stability. Other methods have also been developed for the preparation of "liposome–virus"[51] and "liposome–quantum dot" hybrids.[52] These novel approaches have allowed the use of liposomes in therapeutic, diagnostic and theranostic applications.

There is a wide range of possibilities for preparing liposomes[42] (Table 1). Therefore, depending on the procedure, properties like lamellarity, size, morphology and composition of liposomes can be different. Although most of these processes are standardized and optimized for laboratory scale and preparation is straightforward, there are some limitations like the length of procedures, polydispersity and low drug encapsulation, which are the main drawbacks for conventional liposome preparation methods.

To address these issues, more efficient novel procedures have been described. Conventional liposome preparations are as follows: (a) the thin-lipid film hydration approach was the first methodology developed and the most common method for preparing liposomes.[53] Thus, multilamellar (MLVs), giant unilamellar (GUVs) or small unilamellar (SUVs) vesicles may be obtained which are different from each other according to the preparation technique used.[54,55] However, some limitations can clearly be identified using the technique such as possible liposome degradation upon sonication, heterogeneous size distribution, high temperature exposure or low drug encapsulation efficiencies. (b) Reverse-phase evaporation[56] is another technique that consists of obtaining suspension of large unilamellar vesicles by removing the organic solvent under reduced pressure. This technique involves water-in-oil formation from a mixture of surfactants and lipids as well as an aqueous solution containing the drug. The presence of organic traces in the final formulation could influence the vesicle stability. (c) The solvent injection technique is another conventional method used to prepare liposomes which is based on injection of phospholipids dissolved in an organic

Table 1 Liposome preparation methods and main features

Conventional methods				
Preparation method	Particle size (nm)	Advantages	Disadvantages	Ref.
Thin-lipid film hydration	100–1000	Most widely used method	Low encapsulation efficiencies, sonication, temperature exposure, heterogeneous size distribution	53
Reverse-phase evaporation	100–1000	High encapsulation efficiency	Organic solvent traces	56
Solvent injection (ether or ethanol)	70–200	Ability to control vesicle size	Dilution of liposomes, heterogeneous population, use of high temperature	57

Novel methods				
Preparation method	Particle size (nm)	Advantages	Disadvantages	Ref.
Microfluidic technology	100–300	Synthesis of monodisperse liposomes, high encapsulation efficiency	Fabrication could be complex and needs optimization	58 and 59
Supercritical reverse phase evaporation	100–1200	Environmentally-friendly process, high encapsulation efficiencies	Elevated pressures and temperatures	60
Spray drying	100–1000	Control over particle formation, easily translated to large-scale production	Expense and time-required	61
Membrane contactor technology	~100	Liposomes have homogeneous and small size, high encapsulation efficiency, simplicity for scaling-up	Hydrophilic drug encapsulation needs optimization	62
Crossflow injection	~50	Liposomes of defined size	Vesicle stability due to residual solvent	63

solvent like ether or ethanol into an aqueous phase that contains a particular drug and is maintained at a temperature above the organic solvent boiling point.[57] Although the advantage of this technology is the ability to control the vesicle size, the presence of organic solvent traces in the final formulation, dilution of the liposomes represent a clear disadvantage for this type of approach which reduces the efficiency of this approach.

More recently, novel methods have been described for preparing liposomes. Microfluidic technology has been used for preparing monodisperse liposomes on both laboratory and clinical scales and are easily reproducible.[58,59] Optimizing the parameters such as the flow rate ratio or the size of microchannels provides liposomes with varying and more accurate control sizes. Efficient liposomal distribution, higher encapsulation efficiencies and the reduced loss of reagents are considered as being the advantages of this process. However, this microfluidic device fabrication may be considered a difficult and complex since it requires optimization of multiple fluid inputs and different fluid phases; as a consequence, liposome scale-up production becomes challenging.

On the other hand, there are also techniques that have been developed on an industrial scale which include supercritical reverse phase evaporation,[60] which employs supercritical CO_2 in order to dissolve phospholipids. From an industrial point of view, the use of CO_2 creates an environmentally-friendly process and is a good organic solvent substitute for preparing liposomes as it is non-toxic and not inflammable. Moreover, this technology often yields higher encapsulation efficiencies compared with conventional procedures. Despite these advantages, this method uses elevated pressures (around 20–25 MPa) and temperatures (333 K). Spray drying,[61] membrane contactor technology[62] or crossflow injection[63] techniques are other alternative methods that have also been described for industrial scale-up in liposome production because of their cost efficiency and short-duration processes. The advantages and disadvantages are both covered in Table 1.

Liposome-based technology has revealed serious shortcomings such as instability, a short life and rapid degradation. To address these issues, extensive research has been carried out in order to increase and improve its efficacy. In addition to the synthesis of second generation liposomal drugs described above, the integration of liposomes and polymeric matrices ("liposomes-in-hydrogels" or *lipo-beads*) may represent a promising approach for minimizing burst release and improving tissue localization, especially in topical drug delivery applications including wound therapy and sustained drug delivery. The nature of the polymer may also modify the properties of the encapsulated liposomes (membrane integrity and mechanical stability). This property, together with the liposome concentration, lipid composition as well as liposome interaction with hydrogels, which are also related to the swelling/deswelling properties of the hydrogels, may affect their rheology properties[64] and thereby modulate the release of a therapeutic drug.

The preparation methods described for liposomes-in-hydrogels are mainly governed by incubating the anticipated liposomes with the pre-formed hydrogel beads. Although the encapsulation process of the resultant liposomes within the polymeric matrix takes place and has been experimentally

confirmed, some concerns regarding phospholipids self-assembling and bilayer stabilities should also be considered.[65] This combination has successfully allowed the incorporation of a wide number of liposomes into both synthetic and natural polymer-based hydrogels, obtaining the resultant stimuli-responsive hybrid materials.

Although a number of synthetic polymer-based hydrogels containing liposomes have been described to date,[66–68] many of them may present limitations for clinical applications due to cytotoxic effects. Thus, the use of biocompatible and biodegradable hydrogel matrices has gained increased scientific attention over the last decades. The first liposomal biohydrogel system was described by Weiner and co-workers in 1985.[69] In this work, a liposomal formulation containing two peptide hormones (insulin and growth hormone) was introduced into a collagen gel matrix. Slow release rates of the hormones were obtained when this liposome–collagen hybrid was used. Furthermore, the authors also observed important differences in the release when comparing these liposomal formulations in the absence and in the presence of the hydrogel. Since then the "liposomes-in-hydrogels" strategy has emerged as a promising approach for obtaining self-regulated drug delivery systems.

2. Liposomes in biopolymer-based hydrogels

In principle, the combination of liposomes and biopolymer-based hydrogels could offer important advantages with respect to synthetic polymers for biomedical applications[70] by improving both drug formulation stabilities and drug administration routes.

This review describes the most relevant examples of liposomes encapsulated in biopolymer-based hydrogels for biomedical applications. The use of synthetic polymers[66–68] instead of biopolymers is out of the scope of this review. The manuscript is organized in subsections according to the type of biopolymer used for the preparation of the hydrogel matrix. Particular emphasis is given to the characterization of these hybrid materials and the release profile of encapsulated model drugs or therapeutic molecules (Fig. 1).

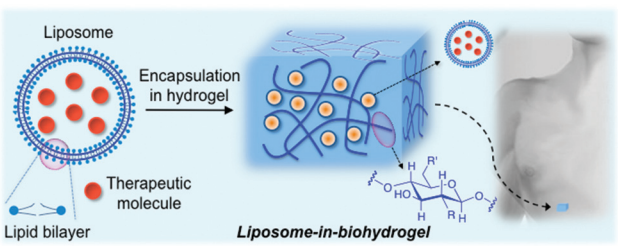
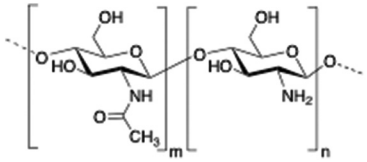


Fig. 1 Schematic concept covered by this review.

2.1. Chitosan



Chitosan is a cationic, biodegradable, biocompatible and mucoadhesive biopolymer composed of randomly distributed β(1 → 4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Chitosan-based hydrogels are one of the most studied systems for applications in biomedical fields such as enzyme immobilization, tissue engineering, wound therapy and drug delivery.[27,71–73]

In 2002, Leroux and co-workers[74] described for the first time the encapsulation of liposomes within chitosan polymeric matrices. Rheological experiments showed that the presence of liposomes in a chitosan-β-glycerophosphate formulation slightly increased both the viscoelastic properties and the gel strength. Additionally, a significant increase in the *in vitro* release time was achieved when liposomes containing model hydrophilic molecules (*i.e.*, 5- and 6-carboxyfluorescein) were embedded in the hydrogel matrix. It is worth mentioning that sustained drug liberation over 2 weeks was obtained with this liposomal formulation. As a control experiment, the time of the release in the absence of liposomes was also evaluated, confirming the complete drug release within 24 h.

Among the variables that can affect control release processes, liposomal composition, vesicle size and surface charge have been studied in order to find a suitable liposomal formulation for application in wound therapy. For example, Škalko-Basnet and co-workers carried out detailed studies using two pH-dependent rhodamine derivatives as model drugs embedded into chitosan-based hydrogels.[75] The results demonstrated that liposome sizes had a minor effect on the *in vitro* drug release whereas both liposomal surface charge and drug lipophilicity considerably affected the release kinetics.

Similar systematic studies have been recently carried out by Ladavière and co-workers.[76] Phosphatidylcholine liposomes made of a mixture of DPPC and a fluorescent lipid at a 99 : 1 molar ratio, were mixed in chitosan solutions (Fig. 2A). This mixture did not affect the final process of the hydrogel formation and rheological studies confirmed that introducing phosphatidylcholine liposomes within the chitosan matrix did not affect the viscoelastic properties of the system either. Additionally, fluorescent liposomes were introduced within the polymeric network and the complexes were characterized by fluorescence and cryo-SEM microscopy. *In vitro* release experiments using carboxyfluorescein as a model drug showed delayed drug release compared to that in the absence of the liposomes (Fig. 2B).

Rouini and co-workers[77] performed both *in vitro* and *in vivo* experiments with a series of radiolabelled liposomes embedded into chitosan-β-glycerophosphate hydrogels. The studied vesicles varied either on the acyl chain length (DMPC,

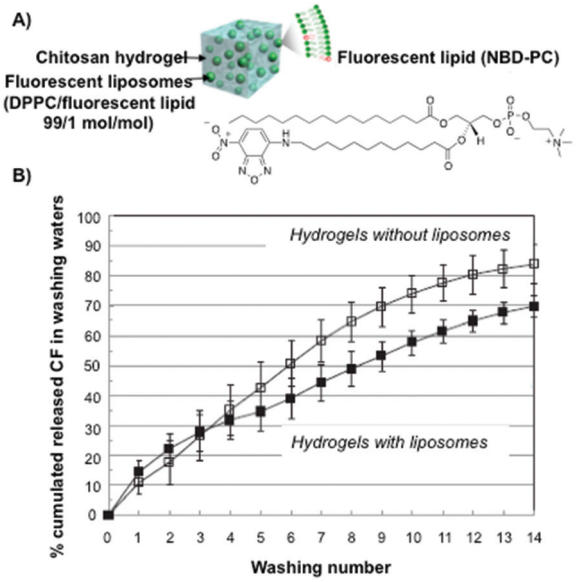


Fig. 2 (A) Schematic representation and the chemical structure of fluorescent liposomes in chitosan hydrogels. (B) Carboxyfluorescein cumulative release percentage for chitosan hydrogels in the absence (open symbols) and in the presence (full symbols) of liposomes. Adapted with permission from ref. 76. Copyright © 2015 Elsevier.

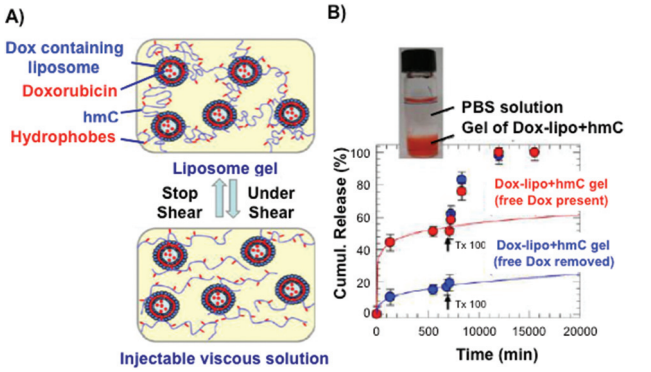


Fig. 3 (A) Pictures of an injectable liposome gel at rest (top) and under shear (bottom). Modified chitosan hydrogels are represented in blue and the grafted hydrophobes in red. (B) Cumulative release of Dox through modified chitosan matrices. Dox release from hydrogels containing liposomes is slower than those in the absence of liposomes. Adapted with permission from ref. 70. Copyright © 2012 American Chemical Society.

DPPC or DSPC) or on the surface charge (DSPG and DOTAP). The authors found that liposomes made of DSPC showed better release profiles than the other ones when injecting the corresponding liposomal formulations in mice models. Furthermore, liposomes made of DSPC showed a higher transition temperature, which generated stronger bilayers than the other liposomes tested. This stability effect induced liposome retention for a long period of time in the peritoneum after injection. An opposite effect was observed for liposomal dispersions made of negatively charged DSPG liposomes. These vesicular carriers left the peritoneum rapidly after injection. Interestingly, the authors were able to prolong this liposomal release at the highest level when entrapping the same vesicular dispersions within chitosan hydrogels. This change in the release behaviour was attributed to the presence of interactions between the matrix polymer and phospholipids.

Besides studying the parameters that can govern control release processes such as size, charge or lipid composition by using model drugs, the use of liposomal formulations entrapped into chitosan hydrogels containing either therapeutic molecules or macromolecules has been successfully reported in the last few years. For example, Škalko-Basnet and co-workers[78,79] were able to entrap Mupirocin, a promising antimicrobial drug, in phosphatidylcholine liposomes of various sizes. Cytotoxicity and both *in vitro* and *in vivo* experiments were also reported. The results confirmed an antimicrobial potential activity against *Staphylococcus aureus* together with an enhancement in wound healing over 28 days *in vivo*. Furthermore, a superior bio-adhesiveness was also exhibited when compared to conventional treatments in burn wound healing rat models.

Ofloxacin, a second-generation fluoroquinolone derivative used for treating severe eye infections, usually suffers major drawbacks (*e.g.*, necessity of frequent administration, low solubility) when administered in solution. However, Hosny and co-workers[80] have recently shown promising therapeutic effects (*i.e.*, prolonged release time and improved stability) when carrying out *in vitro* transcorneal permeation assays by using chitosan-based hydrogels as carriers.

Certain hydrogels are injectable due to the fact that they can exist in a low-viscosity form either prior to or during injection.[81] This property has been applied for site-specific drug delivery applications. Interesting results were obtained in the case of measuring the release kinetics of doxorubicin (Dox), which was encapsulated in the interior of small unilamellar vesicles from a hydrophobically modified chitosan (hmC) gel (Fig. 3).[70] The connection between the liposomes and the hydrogel network was achieved by hydrophobic interactions between polymer chains and liposome bilayers. Steady-shear rheology studies confirmed the properties of injectability of the liposomal Dox–hmC hybrid systems (Fig. 3A). The results showed that the combined system of liposomes and hmC hydrogel could act as an efficient barrier, promoting sustained release of the drug over a week (Fig. 3B).

Recently, Duffy and Ruiz-Hernández[82] have reported the use of chitosan-β-glycerophosphate matrices as carrier systems for local chemotherapy treatment. In this work, a mixture of non-toxic thermosensitive liposomes made of dipalmitoyl phosphatidyl choline (DPPC), monostearoyl-phosphatidyl choline (MSPC) and distearoylphosphatidylethanol-aminepoly (ethylene)glycol (DSPE-PEG2000) at a 85.3 : 9.7 : 5.0 ratio, respectively, was used to entrap Dox (Fig. 4). Interestingly, the cumulative Dox release from the polymer network was enhanced after using hyperthermic pulses, which reduced

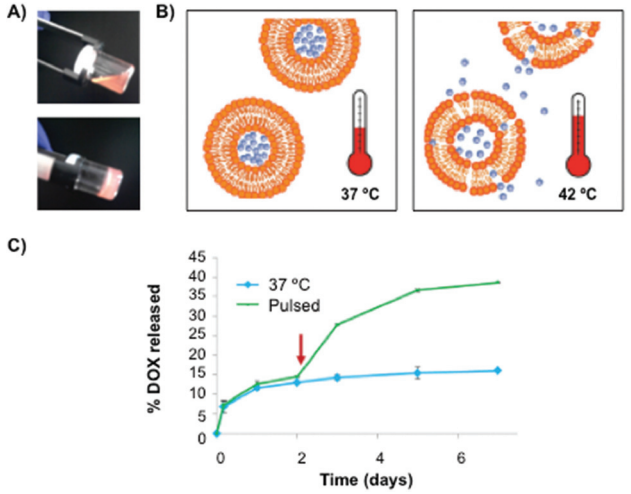


Fig. 4 (A) Pictures of hybrid chitosan hydrogels before (20 °C) and after (37 °C) gelation. (B) Liposome release through the polymeric matrix by using a hyperthermia approach. (C) Cumulative Dox release profiles from the polymeric matrix with and without a 1 h pulse at 42 °C. Adapted with permission from ref. 82. Copyright © 2014 Wiley-VCH.

dsDNA levels when compared to the corresponding non-pulsed samples (Fig. 4B and C).

Besides using chitosan hydrogels for the release studies of low molecular weight molecules, the release of macrobiomolecules has also been studied. In this context, ovalbumin (OVA) and the immunopotentiator Quil A (QA) were entrapped into both cationic nanosized liposomes and cubosomes, a more stable lipid vesicle system.[83] A nice sustained release of the model antigen (OVA) was observed *in vivo* when liposomes were entrapped into chitosan hydrogels. Although OVA-specific antibodies were detected, the system evidenced certain instability even for cubosomes. Unfortunately, OVA and QA in solution forms exhibited similar immunogenicity responses to liposomes when the same thermosensitive chitosan hydrogels were used.

Chitosan-based hydrogels containing liposomes have been further modified by adding additional biopolymers in order to generate novel bio-systems capable of both protecting liposomal formulations and obtaining prolonged, controlled release kinetics. For example, Peptu and co-workers[84] prepared hydrogels made of a mixture of chitosan and gelatin. Both biopolymers were crosslinked with glutaraldehyde and sodium/sulphate tripolyphosphate. A hydrophilic model drug (calcein) was encapsulated in both small unilamellar vesicles (SUVs) and multilamellar vesicles (MLVs) and finally entrapped within the mixture of the aforementioned hydrogels with variable compositions. *In vitro* control release experiments showed improvement in the liposomal stability together with prolonged calcein release from several days to weeks. Interestingly, chitosan/gelatin hydrogels containing MLVs led to better sustained and prolonged release kinetics than those obtained with polymeric matrices containing SUVs. Although the calcein release mechanism from the two biopolymer-based hydrogels is complex, the authors suggested the existence of two steps until the drug reaches the hydrogel surface, consisting of liposome diffusion through the matrix followed by destabilization of the vesicles produced before leaving the hydrogel.

2.2. Gelatin

Gelatin is a biodegradable, thermally denatured protein derived from collagen, a natural protein that is present in mammal tissues, tendons and ligaments. The isoelectric point of gelatine at physiological pH can be modified during its extraction to afford either positively charged basic gelatin (classified as gelatin A) by acidic treatment, or negatively charged acidic gelatin (classified as gelatin B) by alkaline treatment. This versatility has allowed the preparation of numerous polyplexes for drug delivery by combination with a variety of charged biomolecules.[85–87]

There are several examples in which gelatin-based hydrogels have been used for controlled release studies. In most cases, gelatin hydrogels have been chemically modified by adding cross-linking agents such as glutaraldehyde[84] and genipin,[88] among others,[89–91] in order to modify the drug release rates. However, the encapsulation of liposomes in gelatin hydrogels has been only scarcely explored. In 2009, Raghavan and co-workers[92] described the entrapment of vesicles made of sodium oleate (NaOA) in a gelatin matrix at slightly alkaline pH (8.3). It is well-known that these vesicles have the property to self-assemble into spherical micelles in solution at a pH higher than 10 (Fig. 5A and B).[93] Interestingly,

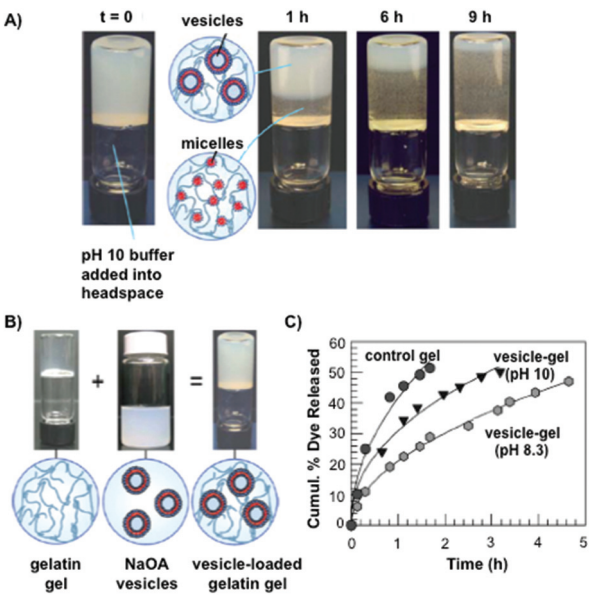
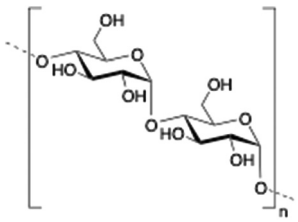


Fig. 5 (A) Micellar movement through gelatin hydrogels due to diffusion of pH 10 buffer. NaOA vesicles are converted into NaOA micelles at different times. (B) Pictures of gelatin hydrogels, NaO vesicles and gelatin-loaded with NaOA vesicles. (C) Calcein release from pH-responsive hydrogels. The release was more gradual at pH 8.3 than at pH 10. Adapted with permission from ref. 92. Copyright © 2009 American Chemical Society.

this pH-responsive property was observed when these vesicles were loaded into gelatin hydrogels at pH 8.3 and 10. When calcein was embedded into the liposomes, a more gradual release was observed at pH 8.3, compared to the control. As expected, this release was accelerated when the pH solution was raised to pH 10 (Fig. 5C). This rapid release was attributed to vesicle-to-micelle transitions, which reduced the transport resistance in the drug diffusion from the hydrogel to the external release solution.

2.3. Dextran



Dextran is a neutral, biodegradable, branched polysaccharide made of glucose molecules composed of chains of varying lengths (from 10 to 150 kDa). From a structural point of view, the native straight chain consists of α-1,6-glycosidic linkages between glucose molecules whereas branches begin from α-1,2, α-1,3 and α-1,4 linkages. Dextran-based hydrogels have been fine-tuned either by adding cross-linking agents such as lactic acid,[94] diisocyanate,[95] polyamines,[96] a mixture of biopolymers[97] or by free radical polymerization using glycidyl methacrylate.[98] These kinds of hydrogels have been explored in a wide variety of biomedical applications such as detection of small molecule drugs[99] and non-viral carriers for proteins.[100]

De Smedt and co-workers reported the coating of hydroxyethyl methacrylated dextran (dex-HEMA) nanogels with SOPC/DOTAP liposomes by either using UV polymerization[101] or by electrostatic interactions between dextran's charged surface and oppositely charged lipid vesicles.[102] DLS and AFM measurements of dex-HEMA nanogels containing lipid particles showed sizes of *ca.* 350 nm. To confirm the presence of the lipid composition in dex-HEMA nanogels, Triton-X100 (TX100) was added to the dispersions in order to solubilize the lipid coating. This resulted in a mixture of naked dex-HEMA and micelles that were also detected by DLS experiments. Alternatively, removal of the lipid coating in dex-HEMA nanogels was corroborated by AFM measurements. As illustrated in Fig. 6, nanogel particles without liposomes (Fig. 6B) displayed a softer surface than those containing SOPC/DOTAP liposomes (Fig. 6A). DLS measurements revealed a relationship between cross-linking density and stability of the particles. Specifically, highly substituted dex-HEMA nanogels showed better stabilities in PBS buffer at 37 °C, which ranged from few days to 2 weeks. However, poorly substituted dextran hydrogels were unstable and degraded quickly. Finally, transfection studies were successfully carried out and confirmed that dex-HEMA nanogels containing SOPC/DOTAP liposomes were able to impart cellular uptake in VERO-1 cells, showing potential as drug delivery carriers (Fig. 6C).[101]

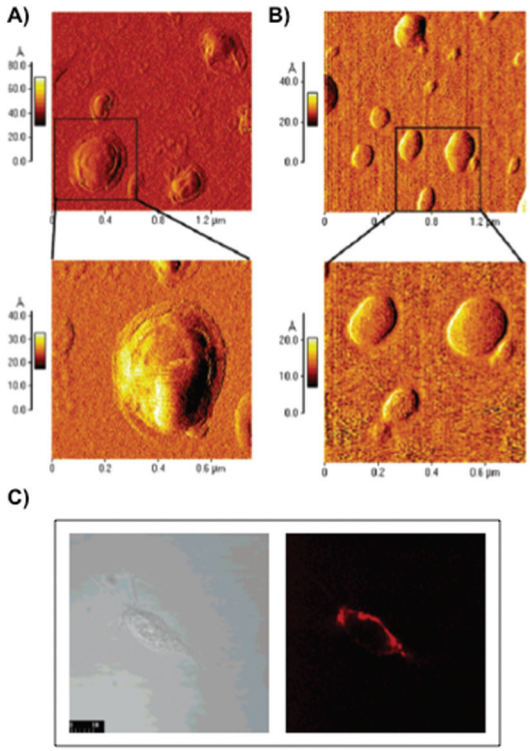
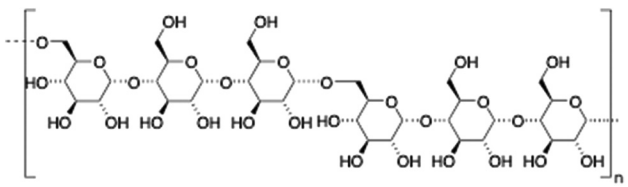


Fig. 6 (A) AFM images of SOPC/DOTAP coated dex-HEMA nanogels. (B) AFM images of native dex-HEMA nanogels when TX100 was added to the dispersion displayed in panel (A). (C) Image of VERO-1 cells incubated with lipid coated dex-HEMA nanogels. Adapted with permission from ref. 101. Copyright © 2005 American Chemical Society.

Another interesting application involving covalently cross-linked dextran-polyethyleneglycol (Dex-PEG) hydrogels was developed by Kros and co-workers.[103] This method is based on the formation of giant unilamellar vesicles (GUVs) by using Dex-PEG hydrogels which were incorporated on glass surfaces *via* a Michael addition reaction. Once Dex-PEG polymer was deposited on the surface, several lipid mixture compositions were added following the film-hydration method by using both PBS and HEPES potassium chloride saline buffers in order to study the effect of ionic strength on the vesicle growth. Interestingly, a nice correlation between Dex-PEG cross-linking density and vesicle size distributions was observed. In principle, the possibility of being able to grow and vary the size of the vesicles could make this system a promising tool to be used in numerous applications such as drug delivery and molecular recognition, among others.

2.4. Pullulan



Pullulan is a highly water-soluble biodegradable polysaccharide made of maltotriose repeating units, containing α-(1 → 4) and α-(1 → 6) glycosidic linkages in a 2 : 1 ratio. This polysaccharide has been widely used in coating applications[104] and drug delivery.[105]

Akiyoshi and co-workers[106] synthesized stable cholesterol-pullulan (CHP) nanogels of *ca.* 30 nm in size based on specific hydrophobic interactions[107] between cholesteryl moieties and pullulan biopolymer in aqueous solutions (Fig. 7).[108,109] So formed CHP particles showed also the ability to form stable hydrogels in the presence of liposomes and using PEG as a cross-linker (Fig. 7A). The hydrogel could help us to increase the colloidal stability, thereby avoiding undesirable aggregation processes. The final CHP hybrid systems were characterized in terms of TEM microscopy and particle-tracking microrheology which indicated that both the strength and the gelation time could be tuned depending on the CHP : liposome ratio. Specifically, liposomal release from CHP nanogels was monitored by coating the aforementioned CHP with liposomes based on a mixture of dimyristoylphosphatidylcholine (DMPC) and cholesterol at a 3 : 1 ratio. The authors observed a sequential dual release profile in which CHP nanogel was first liberated followed by the release of nanogel-coated liposome complexes under physiological conditions, which was fully liberated within 40 days. Interestingly, a clear dependence of the release with the pH was observed between pH 7.4 and 8.0 in which the liposome complex was released within 20 days (Fig. 7B).

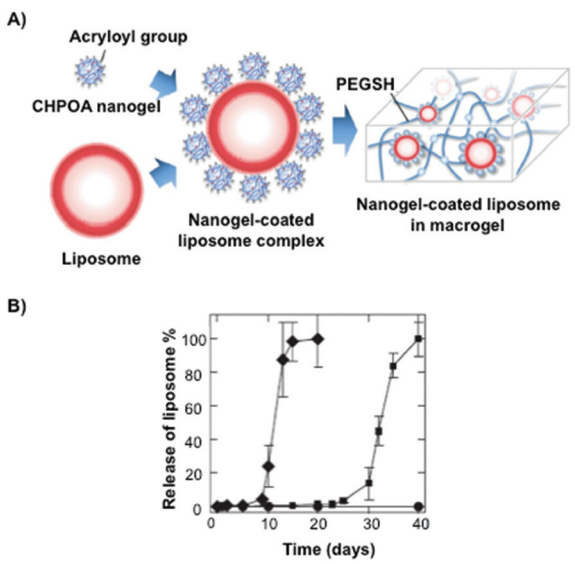
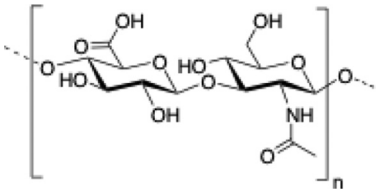


Fig. 7 (A) Illustration of a nanogel-coated liposome complex and PEGSH used as a crosslinking agent. (B) Release profiles of the nanogel-coated liposome from the hydrogel at different pH values (pH 7.4 in squares and pH 8.0 in diamonds). Adapted with permission from ref. 106. Copyright © 2012 Wiley-VCH.

2.5. Hyaluronic acid



Hyaluronic acid (HA) is a linear and negatively charged polysaccharide made of disaccharide repeating units of D-glucuronic acid and *N*-acetyl-D-glucosamine which are linked by β-(1,3) and β-(1,4) glycosidic bonds. HA is involved in several important physiological and biological functions including protein transport[110] and regulation of water homeostasis.[111] Moreover, HA displays anti-inflammatory properties[112] and has been widely used as surgical aid in wound healing.[113] HA has the ability to form robust hydrogel networks and this property has also been exploited, for instance, as a drug delivery system in combination with other biopolymers such as hydroxypropyl methylcellulose (HPMC), polyethylenimine (PEI) or liposomes.[114–117]

Several articles have also described the use of liposomes in combination with HA hydrogels (Fig. 8) for different biomedical applications. For example, the effect promoted by a series of liposomes that depended on their net charge (*i.e.*, neutral, positive or negatively charged), size, lipid concentration and lipid composition was thoroughly studied in HA hydrogels by Agnely and co-workers.[115] The highest viscosity and elasticity in HA hydrogels were observed when liposomes were covered with polyethylene glycol chains. This induced fast immobilization and a long residence time after injection. Interestingly, despite this viscosity detected in these kinds of hydrogels, all formulations were easily injectable by local

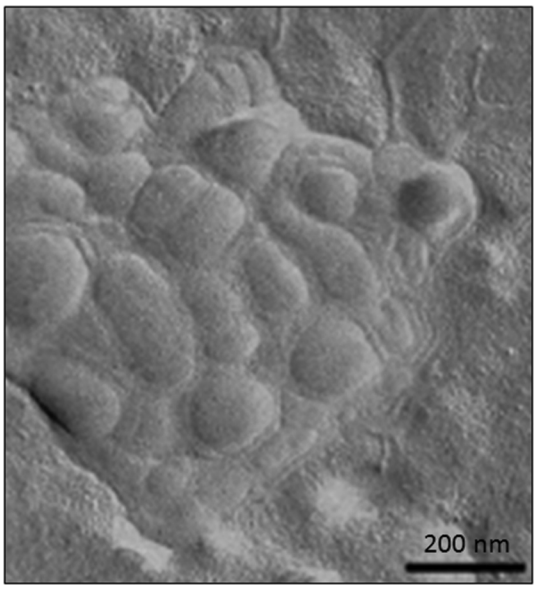


Fig. 8 TEM micrograph of freeze-fractured hyaluronic acid hydrogels containing liposomes. Adapted with permission from ref. 115. Copyright © 2015 Elsevier.

administration due to the shear-thinning behavior of the formulation.

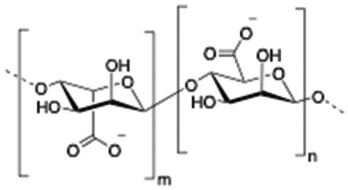
The combination between the HA polymer matrix and liposomes has also contributed to the advance of some disease treatments. For example, Miao and Huang[118] improved osteoarthritis (OA) therapy by combining celecoxib (Clx), an analgesic and anti-inflammatory drug, with a mixture of liposomes (SPC and Chol) that were embedded in HA hydrogels. This formulation showed high encapsulation efficiencies and reduced the adverse effects induced by using Clx alone, such as gastrointestinal toxicities and high risk for cardiovascular events at high doses. *In vitro* experiments confirmed the delay in the liposomal Clx release when liposomes were part of the HA structural framework. These preliminary results were also confirmed *in vivo* showing better effectiveness of the liposomal formulation than current Clx-treatments in both pain control and cartilage protection.

HA is also a natural component of eye tissue, making it ideal as a carrier for ocular drug delivery. In this area, Bochot and co-workers[119] developed a novel formulation consisting of liposomes embedded in HA hydrogels for human uveitis, an intraocular inflammatory disease of the uvea causing loss of vision. In particular, the authors demonstrated that embedding a vasoactive intestinal peptide (VIP), an immunosuppressive agent, within a mixture of liposomes (ratio PG : Chol : PEG : DSPE = 50 : 10 : 35 : 5) remarkably reduced ocular inflammation signs in rats. Although the treatment with VIP loaded in liposomes resulted to be less invasive than surgical implantation, the liposomal formulation leakage could reduce the effectiveness for long-term treatments. However, sustained drug release was clearly improved when liposomal VIP was incorporated into HA hydrogels. This effect was observed in both *in vitro* and *in vivo* experiments with the naked eye, thereby became a promising strategy for local delivery.

In 2013, Venkatraman and co-workers[120] studied the liposomal release of latanoprost (Ltp), a drug used to reduce the ocular pressure, by using two HA-based hydrogels. Before loading the selected liposomes (EPC) in HA hydrogels, the biopolymer was first cross-linked with adipic dihydrazide (ADH) and methacrylic anhydride (MA). The mechanical properties of both systems were controlled by varying the cross-linking degree, which clearly affected the rheological and swelling properties of the hybrid systems. Remarkably, sustained release of liposomal Ltp was accomplished over 90 days with a single administration.

In the area of tissue engineering, Ying and co-workers[121] have recently developed room temperature-liquid injectable HA hydrogels based on a HA–tyramine (Tyr) conjugate containing horseradish peroxidase (HRP). HRP was embedded in a mixture of two thermoresponsive liposomes (*i.e.*, dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidyl choline (DMPC)). Cholesterol was also introduced into the formulation in order to modify the fluidity and reduce the undesirable HRP release at room temperature. The gelation kinetic of the hybrid was found to depend on several factors including lipid content, HA–Tyr concentration, sonication time and composition of the liposome washing solution. Injectability studies were carried out subcutaneously in a mouse model. While the HA–Tyr/H_2O_2/HRP hybrid system remained as a liquid at room temperature, the HRP release upon injection promoted a reaction between HA–Tyr and H_2O_2, inducing the formation of the cross-linked hydrogel scaffold.

2.6. Alginate



Alginate (salt of alginic acid) is an anionic, high molecular weight (500–2000 kDa) polysaccharide comprised of β-D-mannuronate (M) and its C5-epimer α-L-glucuronate (G) residues, which are linked by β-1,4 glycosidic bonds. Alginate has the ability to form hydrogel networks in the presence of divalent cations and other cross-linking agents.[122] A wide number of biomedical applications including wound healing, drug delivery or tissue engineering have been extensively reported.[123]

In 1995, Rudolph and Monshipouri reported the first example in which liposomes were encapsulated within an alginate hydrogel.[124] The authors used dipalmitoyl phosphatidylcholine liposomes as reaction sites for alginate hydrogelation. As a model compound, cytochrome-c was embedded in these large unilamellar liposomes and the release of liposome-encapsulated alginate and calcium alginate beads was evaluated. The authors found rapid drug release for both formulations followed by a slower release rate for alginate beads containing encapsulated liposomes. Around the same time, Yotsuyanagi and co-workers[125] described the factors affecting the loading and release of drug-containing liposomes in alginate beads. The results obtained using 5(6)-carboxyfluorescein (CF) as a model drug in liposomes of egg phosphatidylcholine (EPC) and EPC/cholesterol (EPC/Cho) demonstrated that alginate interacts with the liposomes causing a significant change of permeability. Moreover, the presence of calcium ions seemed to induce a higher drug-leakage than sodium ions. Interestingly, a faster liposome release was observed when using washed gel beads instead of fully-cured beads, showing a proportional relationship between the release and the erosion of the gel at high liposome concentrations.[126] These results suggested that the liposomes were not homogeneous distributed through the gel beads but rather concentrated in the central part. Such dependence between drug release and the erosion of the alginate gel matrix was also observed by Rongqing and co-workers.[127] This group performed *in vitro* and *in vivo* studies on the release of liposomes of L-α-phosphatidylcholine containing bee venom peptide, which were encapsulated into calcium alginate gel beads coated with Eudragit

S100 (methacrylic acid copolymer B). The results demonstrated the potential of this formulation for colonic drug delivery.

In 2006, Wang and co-workers described[128] the use of alginate hydrogel microcapsules for the delivery of bovine serum albumin (BSA) previously encapsulated in multivesicular liposomes (MVLs) *via* a double emulsification process (water-in-oil-in-water). *In vitro* release experiments indicated gradual liberation of the BSA protein over a period of approximately 2 weeks without detecting any burst release effect.

In 2010, Smith and co-workers reported the preparation of alginate-loaded liposomes as a vehicle for the oral delivery of bioactive proteins (*i.e.*, alkaline phosphatase (ALP) as model).[129] The vesicles were prepared from the phospholipid dipalmitoyl phosphatidylcholine using a simple dry film hydration technique. On the other hand, the incorporation of alginate in the liposomes enhanced their stability at gastric pH.

More recently, Štěpánek's group[130] described the preparation of composite microparticles consisting of a calcium alginate gel matrix with embedded liposomes made from cholesterol : DPPC (dipalmitoylphosphatidylcholine) containing 5(6)-carboxyfluorescein. The authors demonstrated the "on-demand" thermo-responsive release from liposomes when embedded within the alginate hydrogel particles. The sensitivity of the thermo-responsive system was found to depend on the DPPC : cholesterol ratio and it was closely related to the phase behavior of the lipid bilayer that formed the liposome.

The same group[131] also prepared hydrogel microcapsules (61 μm) containing fluorescent labelled liposomes having yeast cells that worked as a biological trigger for controlled opening of the microcapsules and consequent liberation of sub-micrometer objects (dye). A similar formulation containing iron-oxide nanoparticles allowed the controlled release of the dye by means of an alternating magnetic field (AMF) (Fig. 9).[132] A temperature increase above the phase transition of the phospholipid bilayer was a necessary condition for the functionality of the system. In spite of high SAR values of the nanoparticles used, the rate of heat loss by conduction from an individual microgel bead was too high to allow any significant temperature rise to take place. The required temperature rise could only be achieved in cases where the beads were more concentrated or accumulated in a small volume.

Kim and co-workers[133] reported the preparation of Janus-compartmental alginate microbeads having two divided phases of sensory polydiacetylene (PDA) liposomes and magnetic nanoparticles. The sensory liposomes were composed of PDA for label-free signal generation and 1,2-dipalmitoyl-*sn*-*glycero*-3-galloyl (DPGG) lipids whose galloyl headgroup has specific interactions with lead(II) forming phenolic–metal complexes, sensing this metal at concentrations as low as 0.1 mM (~20.7 ppm). In addition, about 45% of lead(II) ions could be absorbed by hundred Janus microbeads in 4 h mainly due to the interactions with the carboxylic acids of the alginate matrix (they are around 200 times more present than those of the lipids). The results show the recognition of lead(II) at the PDA liposome surface by DPGG induced distortion of the conjugated yne–ene main chain of PDA, causing a color change from blue to red as well as the development of red fluorescence (Fig. 10). These microbeads have the additional advantage of easy manipulation and convenient collection by applying an external magnetic field.

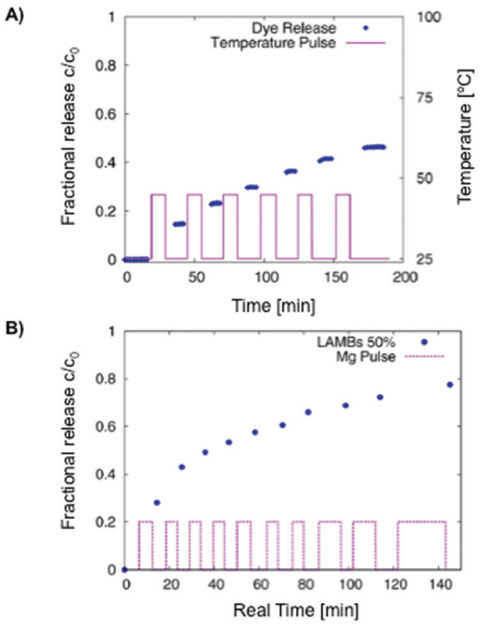


Fig. 9 (A) Carboxyfluorescein diffusion from LAMBs during an on–off temperature program. DPPC : Chol ratio of 2 : 1. (B) AMF-induced release of carboxyfluorescein from LAMBs in water at 50% dilution. Adapted with permission from ref. 132. Copyright © 2013 The American Chemical Society.

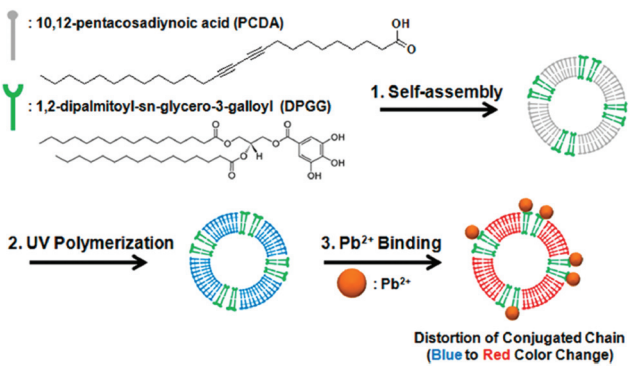


Fig. 10 Co-assembly of PCDA with DPGG to form PDA-DPGG liposomes and a schematic illustration of the colorimetric lead(II) detection. Reprinted with permission from ref. 133. Copyright © 2014 The American Chemical Society.

The release study of a recombinant hepatitis B surface antigen (HBsAg) was investigated by Cohen and co-workers.[134] HBsAg was first encapsulated in liposomes made of a mixture of phosphatidylcholine and cholesterol at a 1 : 1 molar ratio. This carrier was successfully used as an adjuvant for BSA. Liposomes containing HBsAg antigen were confirmed by cryo-

TEM microscopy and were entrapped into alginate-poly-(L-lysine) (PLL) hydrogel polymeric microspheres. Both *in vitro* and *in vivo* experiments showed clear dependency of the liposome release rate on the PLL molecular weight (*i.e.*, liposomes coated with PLL of 214 kDa produced higher liposomal release than PLL of 25 kDa). These findings suggest the efficiency of these hybrids as potential immunoadjuvants because they reduce the amount of antigens and, hence, may eliminate the number of shots necessary for optimal vaccinations.

Oral delivery is another application in which alginate hydrogels have shown to be efficient and robust systems even in the presence of acid environments located in gastrointestinal tracts. This robustness has been recently reported by Yuasa and co-workers.[135] These authors developed a liposomal formulation made of DMPC, cholesterol and non-ionic surfactant tween-20 at a 288 : 72 : 40 molar ratio, respectively, to entrap manganese porphyrin (Mn-por), a superoxide dismutase mimic that maintains the appropriate reactive oxygen species (ROS) levels in cells (Fig. 11A). In order to protect the drug from the gastrointestinal tract acid environment, the liposomal formulation was embedded in alginate hydrogel fibers obtaining hybrids of *ca.* 100 μm in diameter. Although encapsulation efficiencies of Mn-por in liposomes were high (71%), this efficiency diminished dramatically when liposome formulation was embedded in the alginate matrix (3%). *In vitro* experiments confirmed that both alginate hydrogels and liposomes were able to maintain their physical properties under acidic pH, allowing the delivery of Mn-por to the intestine retaining the $O_2^{\bullet-}$ inhibitory activity. The efficacy of this system was also evaluated *in vivo* by monitoring the tumor size in mice (Fig. 11B). Interestingly, there was an inhibition of the tumor growth when alginate fibers containing liposomes were orally administered.

Very recently Deckers and co-workers[136] have described the preparation of barium crosslinked alginate microspheres loaded with temperature sensitive liposomes (TSL/TSL-Ba-ms) for embolization of blood vessels of tumors under mild

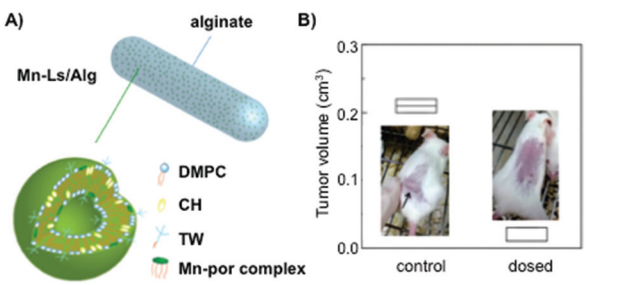


Fig. 11 (A) Mn-Ls/Alg represents an alginate hydrogel matrix containing liposomes with embedded manganese porphyrin (Mn-Ls). (B) *In vivo* anti-tumor efficacy when Mn-Ls/Alg was administered orally. Comparison of the tumor volume in mice transplanted with Colon-26-cells. Adapted with permission from ref. 135. Copyright © 2015 The Royal Society of Chemistry.

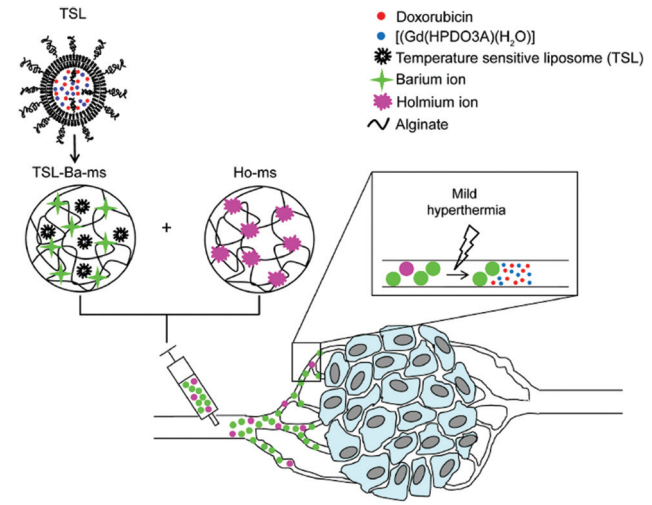
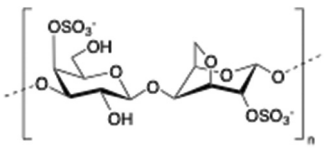


Fig. 12 Schematic representation of temperature sensitive liposomes (TSL) loaded in alginate microspheres crosslinked with barium ions (TSL Bams). The TSLs are loaded with doxorubicin (DOX) and [Gd(HPDO3A)(H_2O)] (T_1 MRI contrast agent). The DOX and [Gd(HPDO3A)(H_2O)] are released from the TSL-Ba-ms during mild hyperthermia. The release of [Gd(HPDO3A)(H_2O)] can be monitored by MRI. Empty alginate microspheres crosslinked with holmium ions (T_2* MRI contrast agent, Ho-ms) are co-injected with TSL-Ba-ms to allow microsphere visualization by MRI. Reprinted with permission from ref. 136. Copyright © 2015 Public Library of Science.

hyperthermia conditions. TSLs contained anti-cancer doxorubicin and [Gd(HPDO3A)(H_2O)] as contrast agents, which were combined with holmium-containing microspheres (Ho-ms) as a tracer agent (Fig. 12). *In vivo* monitoring was carried out in the auricle VX2 tumor of a rabbit after intratumoral injection of a dispersion containing TSL-Ba-ms as well as Ho-ms. The results showed that [Gd(HPDO3A)(H_2O)] was only released after applying mild hyperthermia (42 °C), indicating that the TSLs are stable at 37 °C and do not leak their loading.

2.7. Carrageenan



Carrageenan is a family of linear sulphated polysaccharides made up of galactose repeating units and 3,6-anhydrogalactose (3,6-AG) connected by α-1,3 and β-1,4 glycosidic linkages. Some of these flexible macromolecules such as kappa (κ) and iota (τ) carrageenans can adopt helical structures and promote the formation of hydrogels in the presence of cations. Besides its wide use in food industry as a thickening agent,[137] a good number of biomedical applications[138] have also been described involving carrageenan alone or in combination with additional biopolymers like chitosan,[139] alginate[140] or

gelatin.[141] However, the incorporation of liposomes into carrageenan hydrogels has been scarcely explored to date.

Recently, Kulkarni and co-workers[142] have described the combination of lipid vesicles and κ-carrageenan hydrogels for sustained drug release applications. The strategy involved the loading of a therapeutic drug in a liposomal formulation and subsequent homogenization within the κ-carrageenan hydrogel. The final hybrid system was dehydrated to afford hybrid drug-loaded films (Fig. 13A), which were fully characterized by Fourier transform infrared (FITR) spectroscopy and small-angle X-ray scattering (SAXS). The release kinetics from both native and lipid particles in hydrogel films were investigated and monitored by UV-Vis spectroscopy. The results showed that the drug encapsulation within the hydrogel allowed for more efficient protection of the drug and sustained release in comparison to the native hydrogel in the absence of liposomes (Fig. 13B).

In 2013, El-Menshawe and co-workers[143] developed an alternative to oral delivery systems by using topical administration to improve anti-inflammatory activities and reduce undesirable side effects. Specifically, the authors described the encapsulation of Meloxicam (MX), a non-steroidal anti-inflammatory drug, in niosomes based on a non-ionic surfactant molecule and cholesterol at a 6 : 4 molar ratio. The corresponding niosomal formulation was entrapped into two hydrogels: carrageenan and carboxymethyl-cellulose (CMC) hydrogels. The results of the anti-inflammatory activities *in vivo* clearly showed a decrease in the permeability when increasing the cholesterol molar ratio. Consequently, this enhanced the bioavailability of MX and, thereby, provided better skin permeation.

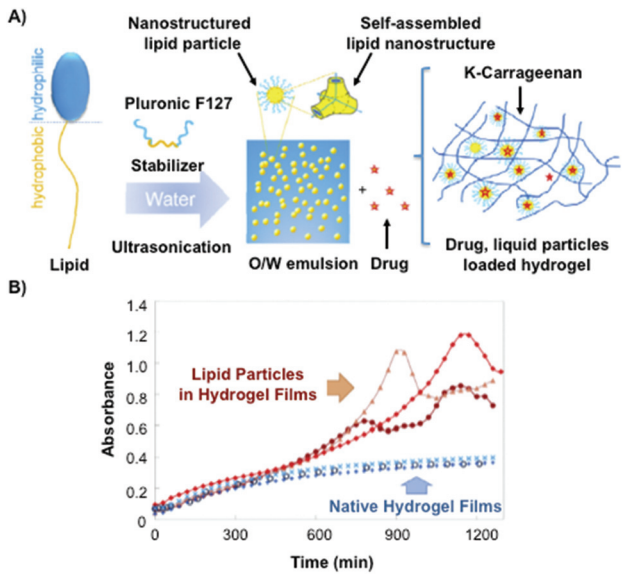
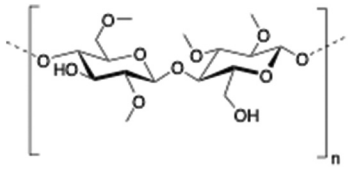


Fig. 13 (A) Lipid molecule made of a hydrophobic and a hydrophilic part is stabilized by using Pluronic F127 upon ultrasonication. This mixture is loaded containing a drug within the κ-carrageenan hydrogel. (B) Release kinetic of the drug was monitored by UV-vis spectroscopy. Drug release from native hydrogel films (blue) and lipid particles within hydrogel films (red). Adapted with permission from ref. 142. Copyright © 2015 Elsevier.

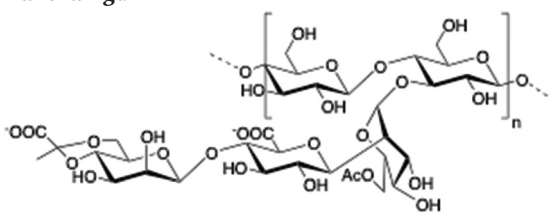
2.8. Methylcellulose



Methylcellulose (MC) is a neutral derivative of cellulose, the most abundant natural polysaccharide composed of (1 → 4) linked β-D-glucopyranosyl units. In MC, methoxy groups substitute the hydroxyls at the C-2, C-3 and/or C-6 positions of anhydro-D-glucose units. MC is able to form hydrogels under isothermal conditions when it is dissolved in hot water,[144] being commonly used in food industry as a thickener and an emulsifier as well as in several biomedical applications.[145,146]

Within the context of this review, Kapadia and co-workers[147] developed a novel approach to deliver acyclovir by using niosomes that were entrapped into a mixture of MC and carbopol. The final goal was to improve the poor bioavailability exhibited by conventional ophthalmic solutions for treatment of herpes simplex keratitis. Thus, acyclovir was encapsulated in two niosomes based on two non-ionic surfactants (span 20 and span 60) and cholesterol at different molar ratios. The results showed that the drug encapsulation efficiency was higher in the case of span 60. *In vitro* release experiments indicated the suitability of the MC hydrogel as an efficient vehicle to deliver the drug in a controlled manner to the site of action due to the presence of bioadhesive polymers in the formulation. Moreover, the use of MC hydrogels containing niosomes helped us to reduce the toxicity that can be observed in conventional therapies.

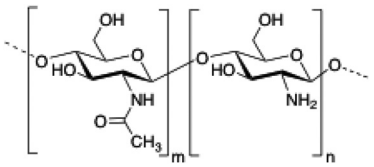
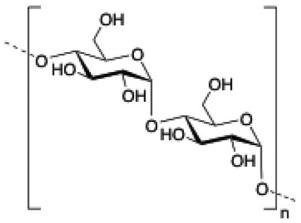
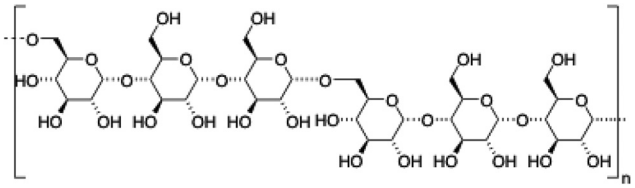
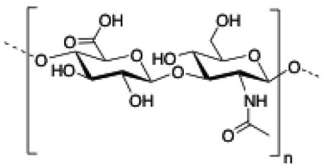
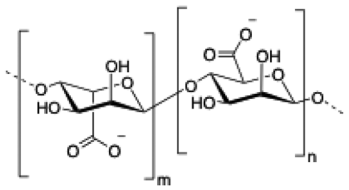
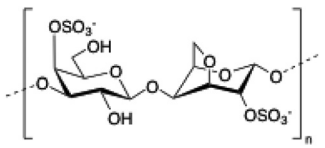
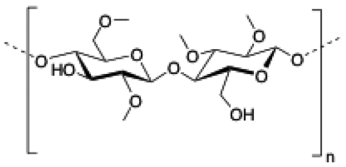
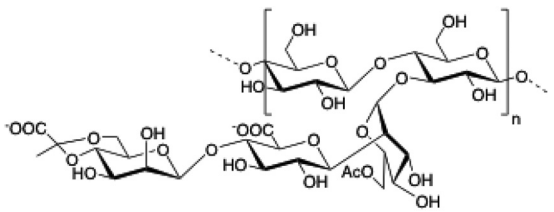
2.9. Xanthan gum



Xanthan gum (XG) is a polysaccharide composed of pentasaccharide repeating units of glucose, mannose and glucuronic acid in a 2 : 2 : 1 molar ratio. Besides its applications in food industry as a stabilizer and thickening agent, XG has also been used in some biomedical applications[148] as a drug release modifier due to its pH-responsive properties.

The mechanical properties of XG-based hydrogels can be modulated upon blending with other biopolymers such as locust bean gum (LBG) in a weight ratio of 1 : 1.[149] The polymeric system LBG/XG was described by Carafa and co-workers[150] as a convenient drug delivery system for ibuprofen and caffeine as model drugs. Firstly, these drugs were encapsulated in non-ionic surfactant niosomes based on Tween-20 and

Table 2 Summary of biopolymers used in the synthesis of liposome-encapsulated hydrogels for biomedical applications

Entry	Biopolymer	Chemical structure	Ref.
1	Chitosan		70 and 74–84
2	Gelatin	Mixture of peptides and proteins produced by partial hydrolysis of collagen	85–93
3	Dextran		101–103
4	Pullulan		106–109
5	Hyaluronic acid		115–121
6	Alginate		124–136
7	Carrageenan		142–143
8	Methylcellulose		147
9	Xanthan gum		149 and 150

cholesterol at a 1 : 1 molar ratio. As expected, a slight increase in size was observed when model drugs were incorporated into niosomes. However, no significant change in the surface charge was detected. Interestingly, a different behaviour in the *in vitro* release was observed from both the niosome-hydrogel matrix and the LBG/XG hydrogel without containing vesicles. Consequently, caffeine, the more hydrophilic drug, was delivered completely and much faster than ibuprofen, which was capable to diffuse only 60% after 48 h. These findings revealed the suitability of niosomes and LBG/XG hydrogels to be used in topical applications due to the slow diffusion of the drugs from the matrix and the protection of the noisome by the hydrogel.

3. Summary and outlook

Biohydrogels (biopolymer-based hydrogels) have found numerous uses across a wide range of food, pharmaceutical and biomedical industries, where their inherent properties of biocompatibility and biodegradability play a key role. In the area of drug release applications, many therapeutic molecules have been successfully entrapped into different biohydrogel carriers (Table 2). However, one of the major concerns is the rapid and uncontrolled release of the drug from the polymer network (burst release), which may induce certain toxicity and undesirable side effects in biological systems. Over the last few years, several strategies have been described to minimize the burst release effect including, among others, the use of liposomes. In this particular approach, and before obtaining the corresponding drug-in-liposome system, a number of parameters need to be optimized such as lipid composition, particle size, morphology and surface charge in order to efficiently encapsulate hydrophilic and/or hydrophobic molecules. Remarkably, the protecting effect provided by liposomes to drugs can be further enhanced through their encapsulation into hydrogel networks. In addition, the possibility of tuning and obtaining either liposomes and/or hydrogels that can respond to environmental stimuli (*e.g.*, light, temperature, pH) makes them ideal candidates for the fabrication of novel drug delivery systems. Several kinds of biopolymer-based hydrogels containing liposomes discussed in this review have proven their efficacy in controlled drug delivery and tissue engineering applications.

These promising results may guide future development of advanced mathematical models of controlled and burst release profiles, providing a platform for the design of more efficient liposomal formulations for biomedical applications.

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