Alginate-based hydrogels for cancer therapy and research

Belen Reig-Vano[1]*, Bartosz Tylkowski[2], Xavier Montané[3], Marta Giamberini[1]

[1] Department of Chemical Engineering, Universitat Rovira i Virgili, Av. Països Catalans 26, Campus Sescelades, 43007 Tarragona, Spain. belen.reig@urv.cat (B.R.); marta.giamberini@urv.cat (M.G.)
[2] Eurecat, Centre Tecnològic de Catalunya. Chemical Technologies Unit, Marcel·lí Domingo s/n, 43007 Tarragona, Spain. bartosz.tylkowski@eurecat.org (B.T.)
[3] Department of Analytic Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Carrer Marcel.lí Domingo s/n, Campus Sescelades, Tarragona43007, Spain. xaviermontanemontanem@gmail.com (X.M)
* Correspondence: belen.reig@urv.cat Av. Països Catalans 26, Campus Sescelades, 43007 Tarragona, Spain.

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Abstract: Cancer is a major health issue concerning to all of us. Current treatment options are still limited due to not-selective action. Encapsulation is contemplated as an innovative approach to address systemic toxicity and tumor resistance caused by traditional therapies, while increasing encapsulated compounds bioavailability. The coating material of capsules strongly determines the success of the system. Since alginate has been proved non-toxic, biocompatible and biodegradable, it is considered a potential vehicle for therapeutic factors encapsulation. Besides, it has the particular ability to form hydrogels, which hold a high-water content and greatly resemble to natural soft tissues. The present review exposes the state-of-the-art and the most sophisticated alginate-based systems for cancer therapy and research. It begins with an overview of alginate hydrogels and the qualities that make them especially suitable for biomedical applications. In the following section, the application of alginate hydrogels as pioneering strategies for cancer treatment is described. Several examples of alginate-based delivery systems of therapeutic drugs, proteins and nucleic acids are provided. Significant emphasis is placed in both oral delivery systems and colorectal cancer therapy. Moreover, the role of alginate 3-D scaffolds for both cell culture and delivery is explained. Lastly, other applications of alginate-based hydrogels such as tumor biomarkers immunosensing and fluorescent surgical marker are included.

Keywords: alginate; cancer; chemotherapy; drug delivery; protein delivery; cell encapsulation; 3-D scaffold.

1. Introduction

Cancer is one of the main causes of mortality around the world. The Global Cancer Observatory database of the International Agency for Research on Cancer, which provides incidence and mortality for 36 cancer types in 185 countries, estimated that 9.6 million people worldwide died because of cancer only in 2018. In addition, 18.1 million people were diagnosed for first time and 43.8 million suffered from recurrent cancer within 5 years or less [1,2]. Lung, prostate, colorectal, stomach and liver are the most prevalent cancer types among men, whereas breast, colorectal, lung, cervix and thyroid cancer are the most frequent types among women. Nowadays one in five men and one in six women are diagnosed with cancer throughout their lives [2]. Unfortunately, one in eight diagnosed men and one in eleven diagnosed women will not overcome this illness [3].

The global cancer burden rises in most of countries. The mortality differences between countries with high, low and medium Human Development Index (HDI) are minor: although incidence rate in countries with low and medium HDI is inferior, the survival rate is also lower due to the late detection and limited access to efficient treatments [3]. Current treatments include surgery, immunotherapy, chemotherapy, targeted therapy, hormonal therapy and radiotherapy [2,4]. In spite of favorable results, the non-selective action of these procedures results in severe side effects which seriously compromise patients' welfare. Tumor resistance

caused by selective pressure is as well a serious drawback that impedes patients total recovery [4]. Hence, oncological research is making huge efforts so as to address these challenges. In this regard, encapsulation represents a promising strategy [5].

Encapsulation is the process of coating solid, liquid or gaseous compounds into a continuous layer or shell, giving rise to beads. Microbeads diameter range from 1 to 1000 μm whereas nanobeads measure from 10 to 1000 nm [6–8]. Beads are called “spheres” if they are spherical matrices with dispersed active compounds within, or “shell/core capsules” if they are composed by a core of a confined compound surrounded by a coating layer or shell (Figure 1) [6–11].

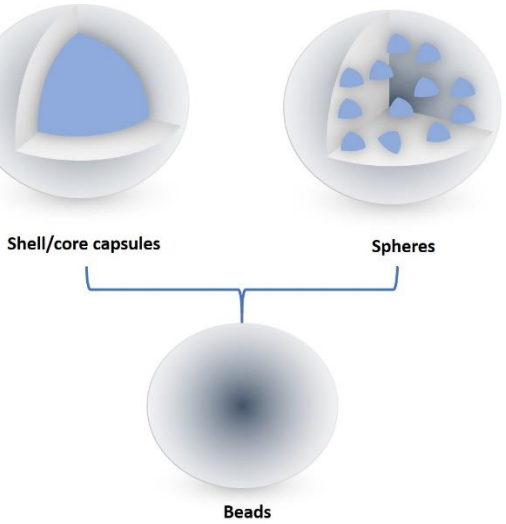

Figure 1: Scheme of beads classification based on encapsulated compound distribution. The active compound is represented in blue. This can be confined in the inner part of the bead forming a core, which is known as shell/core capsule. It can also be dispersed within the bead matrix, which is known as sphere.

Encapsulation allows the controlled delivery of encapsulated therapeutic compounds [2,5,12]. The release profile strongly depends on the matrix composition. Hence, it determines the success of the delivery system. In order to be used for biomedical purposes, coating material has to compile some fundamental requirements, such as suitable physicochemical characteristics and biocompatibility. Synthetic and natural polymers are typically used for encapsulation purposes [13]. Some of the synthetic polymers that have been employed as delivery systems are: poly lactic acid (PLA) [14], polyglycolic acid (PGA) [15], poly(D,L-lactide–co–glycolide) (PLGA) [16], polyethylene glycol (PEG) [17], poly(vinyl alcohol) (PVA) [18,19], polycaprolactone (PCL) [20], poly(beta-amino esters) (PbAE) [21,22] and ethylene-vinyl acetate (EVA) [23]. Natural polymers employed as coating materials include both polysaccharides such as alginate [24], pectin [25], chitosan [26], cyclodextrins [27], dextran [28], agarose [29], hyaluronic acid [30], carrageenan [31], fucoidan [32], starch [33], polyhydroxybutyrate (PHB) [34] and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [35], and proteins such as keratin [36], gelatin [37] albumin [38], collagen [39] and gliadin [7,11,24,40].

Among natural polymers, polysaccharides are especially valuable for biomedical applications because they are abundant in nature and derive from renewal sources; they exhibit a wide diversity of compositions and structures [40]. They are stable, highly water soluble, non-toxic and biodegradable [6,41]. Owing to these properties among others, this review focuses on alginate. An overview of alginate properties and gelation ability is presented in the next sections.

2. Alginate

2.1 Chemical structure

Alginate is the generic nomenclature that refers to a family of linear polysaccharides such as alginic acid and alginate salts, composed by β(1→4) linked β-D-mannuronic acid (M) residues and its C-5 epimer α-L-guluronic acid (G). These monomers are arranged in a non-regular block-wise pattern consisting of homosequences of consecutive M or G residues (M- or G-

blocks) alternated with G/M heterosequences (Figure 2) [42–44]. The composition, molecular weights and hence, material properties of alginate depends on the source of extraction [42–44].

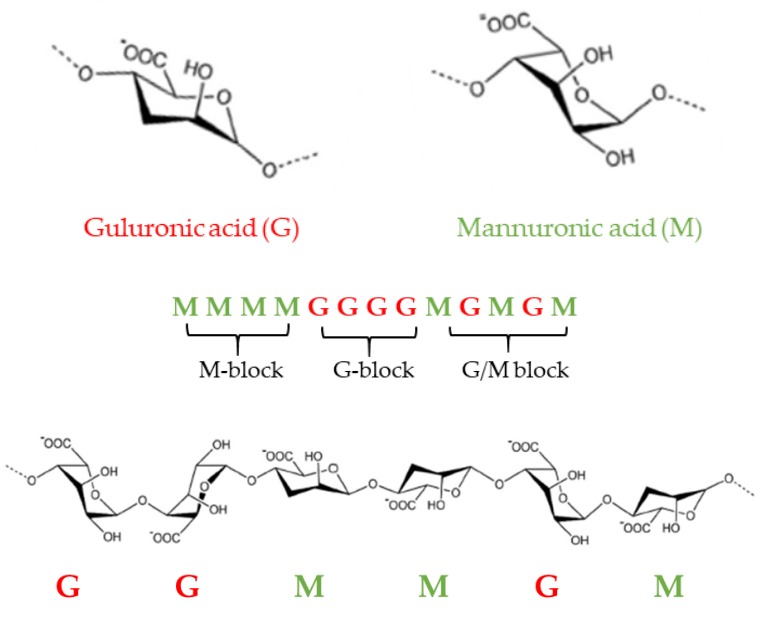

Figure 2: Representative chemical structure of alginate. β(1→4) linked *β*-D-mannuronic acid (M) and *α*-L-guluronic acid (G) monomers and schematic representation of M-blocks, G-blocks and G/M heterosequence.

2.2 Sources and extraction

Alginates are found in a wide range of brown algae such as *Macrocystis pyrifera*, *Laminaria hyperborea, Laminaria digitate, Laminaria japonica, Durvillaea Antarctica, Ecklonia maxima, Lessonia nigrescens, Sargassum spp.* and *Ascophyllum nodosum* [43,45]. Its molecular weight goes from 32 to 400 kg/mol [43]. Variability is mainly due to the specie and algae age [6].

Alginate biosynthesis is as well performed by two bacterial genera: both *Pseudomonas* and *Azotobacter* synthesize alginate as an exopolysaccharide [46]. In *P. aeruginosa*, alginate has a vital role in the configuration of biofilms. Besides, *Azotobacter* employs high G-content alginates as a part of the resistant desiccation structure of cysts [46]. Generally, bacterial biosynthesis provides high quality alginate with better characterized structures and properties [45,47].

Nowadays, more than 200 types of alginate are commercially available [42]. In spite of the heterogeneous composition, most of them come from farmed brown seaweeds.

Algal alginate extraction starts with the removal of counterions followed by the neutralization and solubilization of alginic acid through alginate salts generation [10]. Sodium carbonate or sodium hydroxide are usually employed as alkali compounds, which produce sodium alginate [48]. Once extracted, alginate formulation presents many impurities such as heavy metals, proteins, endotoxins and polyphenols (Figure 3) [45]. These contaminants seriously compromise biocompatibility [43,46]. Ultrapure amitogenic alginates are mandatory for biomedical applications [14]. For this reason, further extraction and rigorous purification processes are necessary.

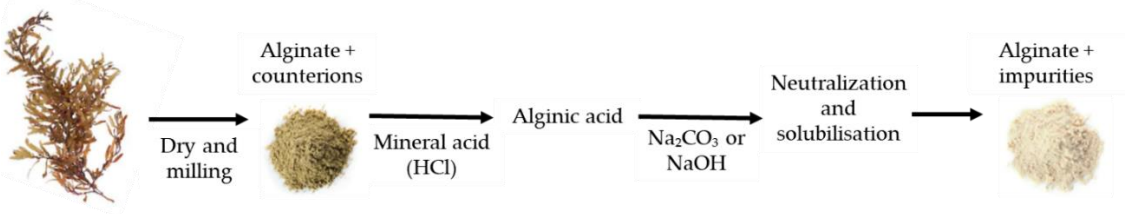

Figure 3: Schema of algal alginate extraction process.

2.3 Alginate gelation

Alginates have the particular gel-forming ability. It can occur either by ionotropic gelation or by acid precipitation [49]. The current review focuses on the former gelation method: ionotropic gelation occurs when negatively charged carboxyl moieties of alginate chains interact with multivalent cations or with cationic polymers [45,50]. Aqueous alginate solution, also referred as "sol" phase, can be cross-linked by both bivalent and trivalent cations [51,52]. Alginate crosslinking gives rise to "sol-to-gel" transition, which results in alginate hydrogels that retain high content of water molecules through hydrogen bonds [53].

Gelation kinetics and features of the obtained hydrogels substantially depend on the ion's properties, such as the valence and radius [49]. Cations employed as crosslinking agents can be arranged according to their affinity to alginate, as follows: Mn < Zn, Ni, Co < Fe < Ca < Sr < Ba < Cd < Cu < Pb [49]. Alginate interaction with trivalent ions such as Fe and Al is stronger than with divalent ions [49].

The interaction between multivalent cations and alginate is described by the "egg-box" model. It suggests that two G-blocks of adjacent chains form electronegative cavities - "egg box junctions", which are able to host cations [47]. Normally, "egg box junctions" are occupied by alginate counter-ions, such as sodium in case of sodium alginate [10]. Nevertheless, these can be replaced by multivalent ions whose size and charge also fit in the cavities [50]. As a result of the ion exchange, the "egg box" structure forms (Figure 4) [47]. Although just G-blocks were considered to be involved in intermolecular crosslinking [42], MGM-blocks may also contribute to gelation through weak interactions with cations [45,48].

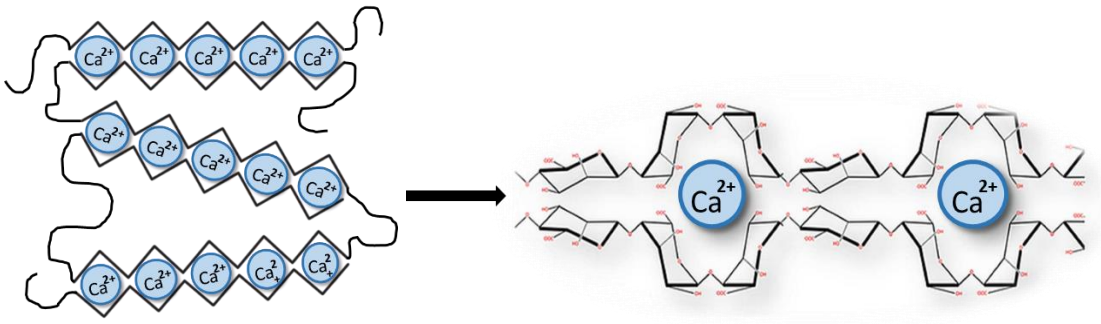

Figure 4: Schema of alginate "sol-to-gel" and "egg-box" model.

Ionotropic gelation may occur *via* external or internal mechanisms [6]. The difference lies in the source of crosslinking agent and the gelling kinetics (Figure 5). External gelation is based on the diffusion of an external source of multivalent cations. Hydrogels are produced when the alginate "sol" phase, usually in form of droplets, is introduced into an aqueous crosslinking solution, which is commonly a soluble salt such as calcium chloride (Figure 5a). Crosslinking ions quickly diffuse into the "sol" phase. As a result, cations distribution within the gel is heterogeneous, being less concentrated in the inner parts of the droplets. This gradient is controlled by the diffusion rate of the crosslinking agent [6,48,49].

Internal gelation mechanisms involve the incorporation of an inactive form of the crosslinking agent within the "sol" phase. For example, insoluble calcium salts such as calcium oxalate, tartate, phosphate, carbonate and citrate are usually used as internal calcium source (Figure 5b). Cations release and crosslinking activation is performed through controlled alterations of the system properties such as pH fluctuation or ion solubility [48]. Hence, homogeneous gels with uniform ion distributions are obtained [10]. However, they are often soft and prone to be clustered [24,54].

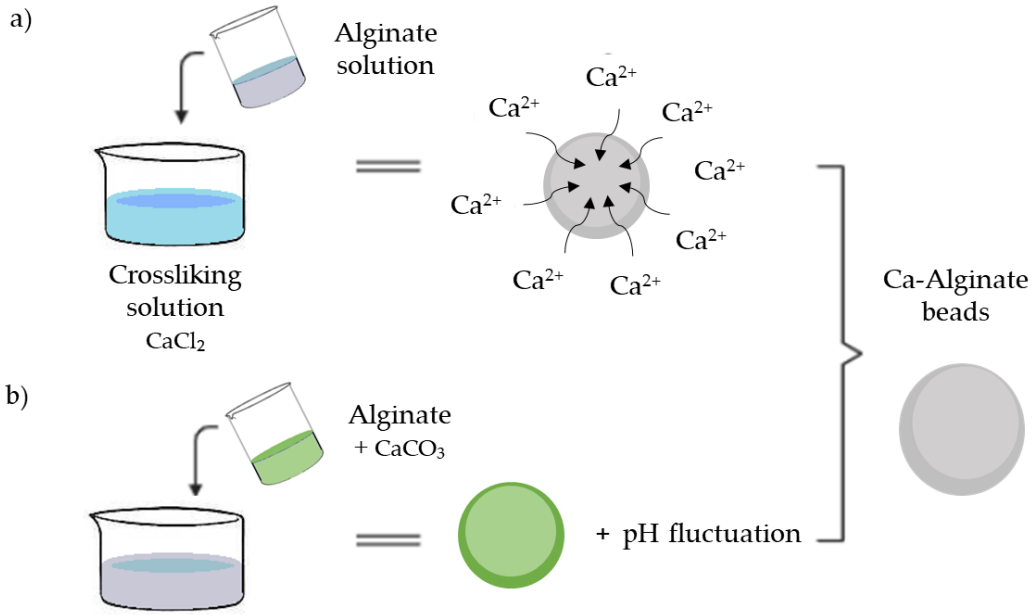

Figure 5: Schematic representation of (a) external and (b) internal gelation

Alginate can also interact with oppositely charged polyelectrolytes and produce hydrogels [41]. Electrostatic attraction between the anionic groups of alginates and the cationic groups of polycations leads to polyelectrolyte complexation. This approach is usually coupled with ionotropic gelation [44]. Crosslinking cations reinforce the polyelectrolyte complex [6].

Beads obtained through polyelectrolyte complexation can be produced *via* layer-by-layer deposition (LbL) (Figure 6). It results in alternated layers of oppositely charged polyelectrolytes sequentially arranged which surround the core material [55]. Some polyelectrolytes that have been applied in combination with alginate are chitosan [56] and poly-L-lysine [57].

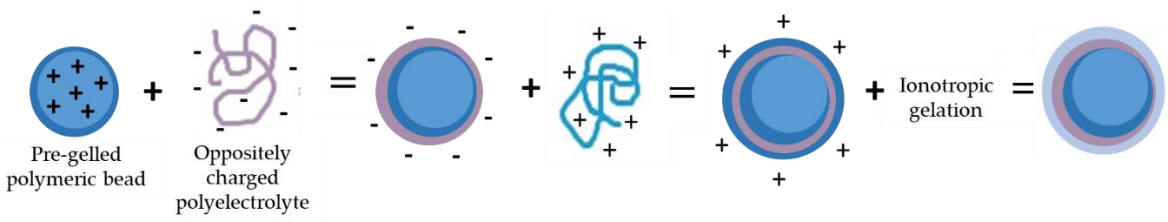

Figure 6: Schematic layer-by-layer deposition

Ionotropic gelation is a simple, fast and cost-effective process. It is performed under mild conditions [6,24,44]. For polyelectrolytes complexation, complexation promoters are not needed and thus, further purification processes are not required [6].

Alginate gelation may occur as well by photo-crosslinking [58], gamma irradiation [59] or by a combination of these methods with ionotropic crosslinking. In addition, alginate can be covalently crosslinked by means of agents as glutaraldehyde [60,61] or poly(ethylene glycol)-diamine [62]. However, these are not as safe as ionic crosslinking for biomedical applications [45].

3. Alginate hydrogels application in cancer therapy

3.1 Benefits of alginate for biomedical applications

Alginate has been applied by many industries such as food, textile and paper-printing, as well as in agriculture, aquaculture and industrial waste-water treatment [8,49,63,64]. For example, recently it was patented the encapsulation of fungal microsclerotia such as *Metarhizium* spp.

within alginate. The patent 16/960413 teaches that alginate enhances the efficacy and stability of encapsulated entomopathogenic fungi towards a variety of insects.

Alginate has been employed as well in fish and crustacean's vaccines. The invention KR2019/017275 discloses the formulation of an acid-treated low-molecular-weight alginic acid and an attenuated microorganism for oral vaccination. It addresses issues such as the improvement of water quality in farms and costs lowering. Also the patent 16/062445 teaches several methods for removing organic acid anions, heavy metal ions and thermally degraded organic products from a liquid by means of calcium alginate-based adsorbents.

In addition, the patent WO/2020/118080 discloses a novel alginate-based fiber. This is made of alginate, cellulose and a polyol plasticizer, such as glycerol, and it is an innovative alternative to petrochemical based textile polymers.

Certainly, alginate is particularly valuable for the biomedical field. For example, it has been used to provide dressings with antimicrobial properties. The patent EP3660191A4 discloses a novel method to manufacture antimicrobial alginate fibers. It overcomes technical challenges such as the cancellation of active compound's antimicrobial activity.

Furthermore, the United States Patent 9421220 discloses an alginate-based anti-cancer formula for the treatment of colorectal cancer. It comprises an iron chelator and allows selective therapeutic action. Other innovative anti-cancer formulation is described in the patent KR2020/004875. It teaches low molecular weight alginic acid as active ingredient together with an anticancer adjuvant as a method for preventing or treat cancer.

Not only is alginate employed as therapeutic agents' carrier, but also as 3-D scaffold for tissues engineering and cell culture substrate, among others. For instance, the invention US2020/031877 provides a thrombin-free plasma-alginate gel to support stem cell growth. The material includes fibrin, alginate and calcium. Also modified alginates have been patented for pancreatic islet cells encapsulation, especially useful for diabetes treatment. Besides, the patent CN2018/109187 discloses a formulation based on alginate hydrogel so as to preserve cellular activity of cell solutions during storage at low temperatures. The preservation solution is composed by sodium alginate, calcium chloride and hyaluronic acid.

Doubtlessly, alginate presents many advantages that make it especially useful for therapeutic agents' encapsulation (Figure 7): it is abundant in nature and derive from renewable sources [45,47]; it is inexpensive and commonly available [24,53].

Owing to the hydroxyl and carboxyl groups of each G and M residue, alginate is highly reactive. Chemical functionalization has been widely explored and a wide variety of derivatized alginates is available, which enables tailoring hydrogels properties accurately for specific applications [43,47].

Alginate meets the safety requirements concerning to biocompatibility, biodegradation and toxicity. Given its inert nature, it is not irritant, toxic nor immunogenic [8,24,42,47,49]. Although the human organism does not synthesize specialized enzymes for it, alginate biodegradation has been proved [8,24,42,45] . What's more, disintegration does not cause any harmful effect on genetic information nor cytotoxicity. Alginates whose molecular weight is lower than 50 kDa are effectively removed by kidneys [44].

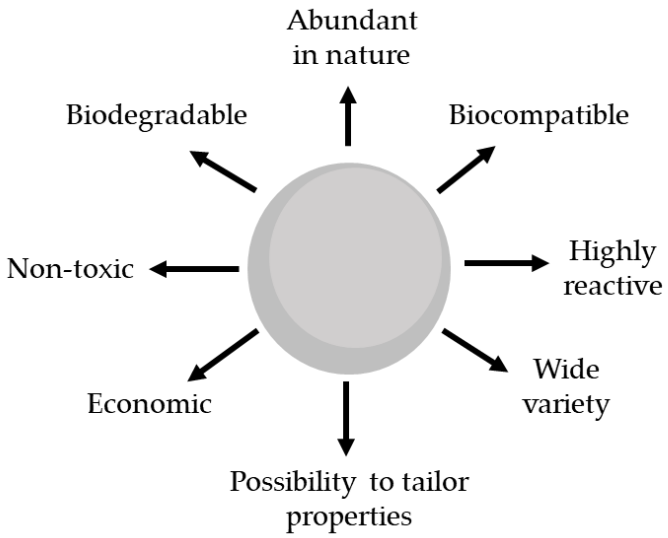

Figure 7: Benefits of alginate for biomedical applications

3.2 Alginate-based hydrogels as delivery system of anticancer therapeutic compounds

Alginate beads protect encapsulated compounds from the environment and improve their bioavailability [45,65]. Encapsulation enables the sustained and local delivery of loaded factors. Therefore, higher concentrations are achieved in targeted parts of the organism with minimal side effects at undesired places.

Ionotropic gelation is the most employed method to produce alginate capsules [43,46]. Hence, encapsulation takes place in an aqueous medium, under mild conditions and with non-toxic solvents involved [66]. Calcium is the most commonly used cross-linking agent.

Encapsulated compounds are released through matrix degradation or diffusion. The delivery rate rises when alginate matrix swells. For instance, sodium alginate hydrogels cross-linked with $CaCl_2$ swell in the presence of Na^+ ions; afterwards ion exchange occurs and electrostatic repulsive forces increase. Hence, surface pore size extends and release rate boosts [24,67,68]. Neutral and basic pH values also produce beads swelling and destabilization. On the contrary, acid pH values yield to alginate carboxylic moieties protonation and beads shrinkage. Most of the times, the release mechanism is a combination of diffusion and matrix degradation [24]: when beads matrix swells, active compounds diffuse and surface dissolves.

3.2.1 Alginate-based hydrogels as drug delivery systems

Recent studies aim to encapsulate chemotherapeutic drugs within alginate matrix following different methodologies. For instance, Kwon et al. prepared redox-responsive cystamine-loaded alginate microspheres by emulsification followed by ionotropic gelation (Fig. 8a) [69], whereas Lin et al. encapsulated astaxanthin (AST), which is a carotenoid with powerful anti-oxidative and anti-inflammatory properties [70], by means of extrusion followed by ionotropic gelation (Fig. 8b) [71].

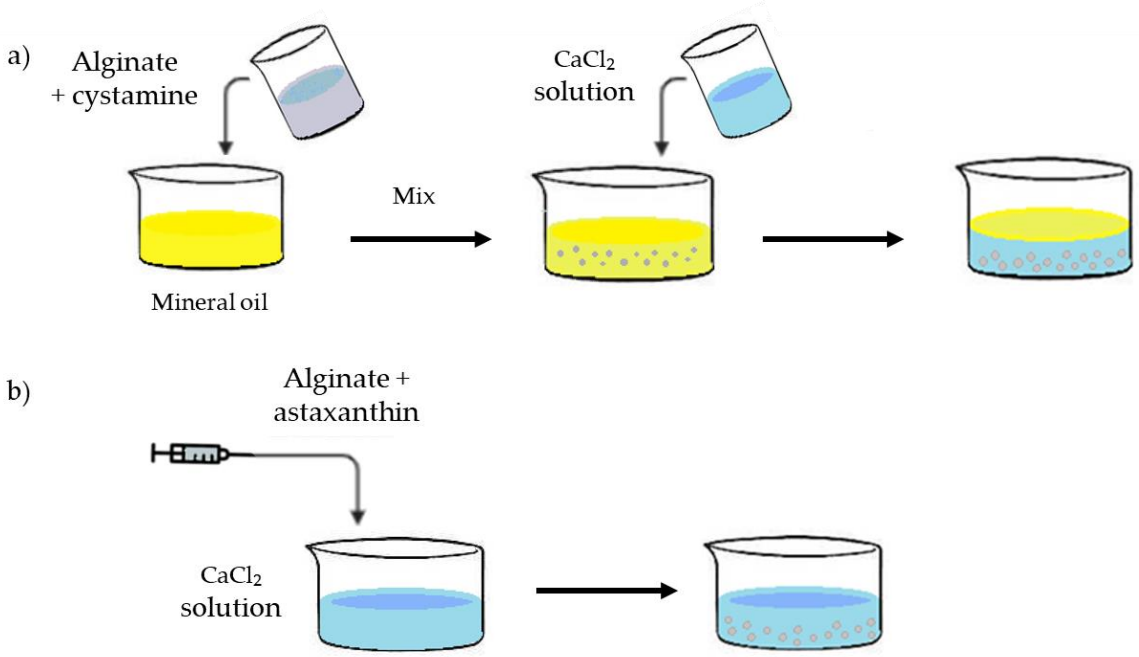

Figure 8: Scheme of beads production through (a) extrusion and (b) emulsification method

The methodology to encapsulate the same drug may differ too. For example, not only has AST been encapsulated through extrusion [71] but also, by double emulsion. Such is the case of Zhang et al. who employed this technique so as to produce astaxanthin-loaded calcium alginate microspheres [72]. The obtained beads demonstrated the efficient inhibition of hepatoma HepG2 cancer cells. Generally, the size of beads obtained though extrusion is considerably larger than when obtained by emulsification-gelation.

Among other chemotherapeutic drugs that have been encapsulated within alginate matrix is arsenic trioxide (AOT), which is usually employed for the treatment of acute promyelocytic leukemia and advanced primary liver cancer. Lian et al. prepared AOT-loaded alginate capsules by ionotropic gelation and further coated them with red blood cell membrane [73]. *In vivo* studies with human liver cancer SMMC-7721 cells-bearing mice pointed the relieve of AOT systemic toxicity while anti-tumor effects improved.

Besides, Boi et al. encapsulated doxorubicin hydrochloride (DOX)-loaded microvoids delimited by a dextran sulfate sodium salt and poly-L-arginine hydrochloride within alginate hydrogels through extrusion followed by ionotropic gelation [51]. DOX is a widely employed antitumor agent for the treatment of diverse solid tumor types such as leukemia, aggressive lymphoma, ovarian, breast, stomach, lung, and bladder [74]. The capsules' cytotoxicity was proved on human breast adenocarcinoma MCF-7 cells. With this approach Boi et al. achieved a sustained and slower release rate compared to free DOX-loaded alginate beads.

Drug release kinetics highly depend on the interaction between alginate and the loaded therapeutic compound, which is governed by electrostatic forces [63]. Given the anionic nature of alginate across a wide range of pH values, it effectively interacts with positive charged molecules. Although encapsulation of anionic drugs is also possible, they are faster released [49]. If chemical interactions are weak, the release rate is mainly controlled by the nature of the therapeutic agents [47]. Generally, hydrophilic compounds diffuse quicker though the alginate matrix than hydrophobic compounds [40,63]: the reason is that hydrophobic drug-loaded alginate beads do not swell as much as hydrophilic ones do. For this reason, some encapsulation strategies include alginate modification so as to improve the affinity for the loaded active compound. For example, Jia et al. prepared DOX-loaded nanoparticles based on PEGylated oxidized alginate crosslinked with fluorescent carbon dots [75]. They aimed at pH-responsive DOX delivery in acidic environment. DOX was linked to PEGylated oxidized alginate nanoparticles through acid-labile "Schiff-Base" conjugation. Cytotoxicity and cellular uptake assays with ovarian cancer SKOV3 cells evidenced pH-triggered and nucleus-targeted DOX delivery. Thus, DOX anticancer effects were enhanced. Moreover, the fluorescence

competence of the nanoparticles permitted their application for imaging-guided drug delivery. Besides, Talebian et al. modified alginate hydrogels in order to achieve affinity-controlled release of DOX and gemcitabine [76]. Dopamine-modified alginate was part of the core and improved chemotherapeutic agents' affinity, whereas the shell was composed by methacrylated-alginate. A slower drug release was evidenced compared to unmodified alginate delivery system. DOX- and dual-loaded capsules considerably inhibited tumor growth of pancreatic cancer cell lines MIA PaCa-2, PANC-1, and BxPC3. As well Rezk et al. conjugated alginate with polydopamine [77]. They aimed at pH-responsive delivery of bortezomib as drug release studies confirmed.

Alginate has been also modified in order to promote targeted therapeutic effect. For instance, Sarika et al. produced galactosylated alginate-curcumin micelles to enhance hepatocytes affinity [78]. Curcumin is a polyphenol with a well-known anti-inflammatory and antioxidant capacity due to its potent free radicals scavenging activity [79]. Results pointed the galactose moiety to enable a selective curcumin release to human liver carcinoma HepG2 cells. Alginate functionalization improved curcumin cellular uptake, accumulation and targeted cytotoxic effects against HepG2 cells.

Despite anticancer drug-loaded alginate hydrogels are effective delivery systems, additional materials often constitute the alginate-based matrix. Alginate can complex other polyelectrolytes such as polysaccharides and proteins. For example, Sohail et al. studied the feasibility of chitosan and alginate capsules for the delivery of amygdalin [80]. Chitosan is a natural polysaccharide that has been widely applied for encapsulation purposes both alone and in combination with alginate and other components. When amygdalin is hydrolyzed by glucosidases, it gives rise to hydrocyanic acid, which has both antitumor and anti-inflammatory properties [81]. In this study, beads were prepared through LbL technique (Figure 6). Assays with lung cancer H1299 cells evidenced an increased amygdalin cellular uptake and cytotoxicity.

Further investigation teams have employed chitosan-alginate formulations for the encapsulation of anticancer agents. Such is the case of Alsmadi et al. who produced a delivery system for the intratracheal administration of cisplatin (CDDP) [82]. CDDP is a chemotherapeutic drug that comprises a platinum atom. In spite of its high toxicity, it has been employed for several years and it is still in use. *In vivo* toxicity tests demonstrated the success of the CDDP delivery system. Both the CDDP toxicity and the mortality rate of rats greatly decreased after intratracheal administration.

Apart from chitosan, other natural polysaccharides have been used together with alginate for chemotherapeutic drugs encapsulation. For instance, Hassani et al. employed Gum Arabic for the encapsulation of curcumin [83]. The anticancer capacity of the system was characterized in diverse cancer cell lines: human liver cancer HepG2, human colon cancer HT29, human breast cancer MCF-7 and human lung cancer A549 cells. Results pointed the higher toxicity of curcumin-loaded beads compared to free curcumin. In addition, Upadhyay et al. prepared microbeads based on a formulation of locust bean gum and sodium alginate [52]. In this assay, aluminum chloride was employed as the cross-linking agent. Beads were loaded with capecitabine. The formulation was further optimized for the treatment of colonic tumors. Pharmacokinetic parameters improved after oral administration to healthy rats. Drug release occurred in a controlled manner. Cytotoxicity assays with human colon cancer HT-29 cells confirmed a higher reduction of tumor growth compared to free capecitabine.

Synthetic polymers have also been applied together with alginate for encapsulation purposes. Such is the case of PVA, which was used to encapsulate green tea polyphenols [84] and 5-fluorouracil (5FU) [85] by Chen et al. and Dalei et al. respectively. Besides, Hosseinzadeh et al. designed a brain implant made of temozolomide (TMZ)-loaded PLGA microspheres embedded within an alginate matrix for glioblastoma therapy [86]. This would enable the precise location of the delivery system at the tumor site and the sustained TMZ release. The implant – GlioMesh was produced by means of microextrusion 3D-printing technology and ionotropic gelation. Cellular viability tests on glioblastoma U-251 MG and U-87 MG cells, western blotting and mitochondrial damage evaluation evidenced the greater efficiency of the novel delivery system over free TMZ.

A different strategy involves alginate complexation with proteins. In 2020 Elbialy et al. prepared capsules of DOX-loaded caseinate nanoparticles with alginate shell [87]. The presence of casein makes possible the encapsulation of both hydrophobic and hydrophilic drugs. Although free DOX is barely internalized by cancer cells, alginate-coated beads ameliorated anticancer effectiveness of DOX in Ehrlich carcinoma-bearing mice.

Moreover, alginate have been employed in combination with both polysaccharides and proteins. For instance, Shen et al. employed alginate-chitosan multilayers as coating material of DOX-loaded bovine serum albumin (BSA)-gel-capsules [61]. BSA enables pH-sensitive drug delivery since it triggers DOX release just in acidic environments, such as tumor microenvironment. Alginate-chitosan DOX-loaded BSA-gel-capsules were injected within the tumor of MCF-7/ADR cells xenografted mice, a breast cancer model. Both DOX concentration and retention time at the injection site improved.

Alginate allows smart delivery, which is the controlled release of encapsulated factors triggered by a stimulus. This can be a pH fluctuation [61,77,88,89], a particular temperature [90], a magnetic field [91,92] ultrasound [93] or redox potential [69]. For example, Xing et al. employed $CaCO_3$ nanoparticles, which are especially sensitive to pH, as intelligent carriers [88]. These were loaded with DOX and coated with both alginate and chitosan by means of LbL technology. *In vitro* studies with human cervical cancer HeLa cells evidenced a pH-sensitive DOX release. Besides, Rezk et al. employed alginate polydopamine hydrogels to achieve pH-responsive bortezomib (BTZ) delivery [77]. They took advantage of the dissociation of catechol group that bound polydopamine to BTZ at acidic pH, to control the precise BTZ release at tumor's microenvironment. Release profile studies proved the success of the delivery system.

Wei et al. also prepared a smart delivery system composed by DOX-loaded gelatin core and alginate-polydopamine (PDA) shell, which has a strong photothermal conversion effect [90]. The effect of the hydrogels on breast cancer 4T1 cells was studied. When irradiated with near infrared light, PDA increased core material temperature and promoted DOX diffusion. In addition, Yun et al. combined sodium alginate with graphene oxide for the development of an electro- and pH-sensitive drug carrier [89]. The loaded drug was methotrexate. *In vitro* release kinetics studies proved the electro- and pH-responsive methotrexate delivery.

Magnetic beads suppose an attractive approach for controlled drug release since they enable a precise and on-demand delivery by means of a magnetic field. For instance, Song et al. employed Fe_3O_4 as the magnetic compound, together with alginate and chitosan multilayers, for the targeted delivery of curcumin [91]. Therefore, higher cellular internalization and cytotoxicity were achieved on breast cancer MDA-MB-231 cells compared to free curcumin. Moreover, Amani et al. included to the magnetic curcumin-loaded alginate Fe_3O_4 composite, BSA and poly((3-acrylamidopropyl)trimethylammonium chloride) [92]. Curcumin stabilization was achieved as well as a controlled drug release under acidic pH.

Besides, Baghbani et al. coated with alginate multifunctional perfluorocarbon nanoemulsions; namely, perflourohexane (PFH) [93]. Not only act perfluorocarbon nanoemulsions as ultrasound-responsive passive-targeted carriers but also, as ultrasound-responsive imaging contrast agents. DOX-loaded alginate-stabilized PFH nanodroplets were prepared through nanoemulsion followed by ionotropic gelation. Ultrasound responsivity assays evidenced the DOX release triggered after sonication with 28 kHz therapeutic ultrasound. *In vivo* studies with breast cancer 4T1 cells-bearing mice demonstrated the total tumor elimination.

Sometimes, alginate-based delivery systems integrate different therapeutic agents. For example, Wu et al. produced a hydrogel through the combination of previously cross-linked composites (Figure 9) [94]. One composite included peptides (Pept) and cisplatin (CDDP) whereas the other comprised alginate (AlgNP) and irinotecan (IRN). The resulting hydrogel aimed at the differential release of CDDP and IRN. Its effectivity was tested in pulmonary adenocarcinoma A549 cells and in A549 cells xenografted mouse model. Temporal dual drug release was achieved. This strategy supposes a great improvement for combination therapy since it promotes synergistic therapeutic effects.

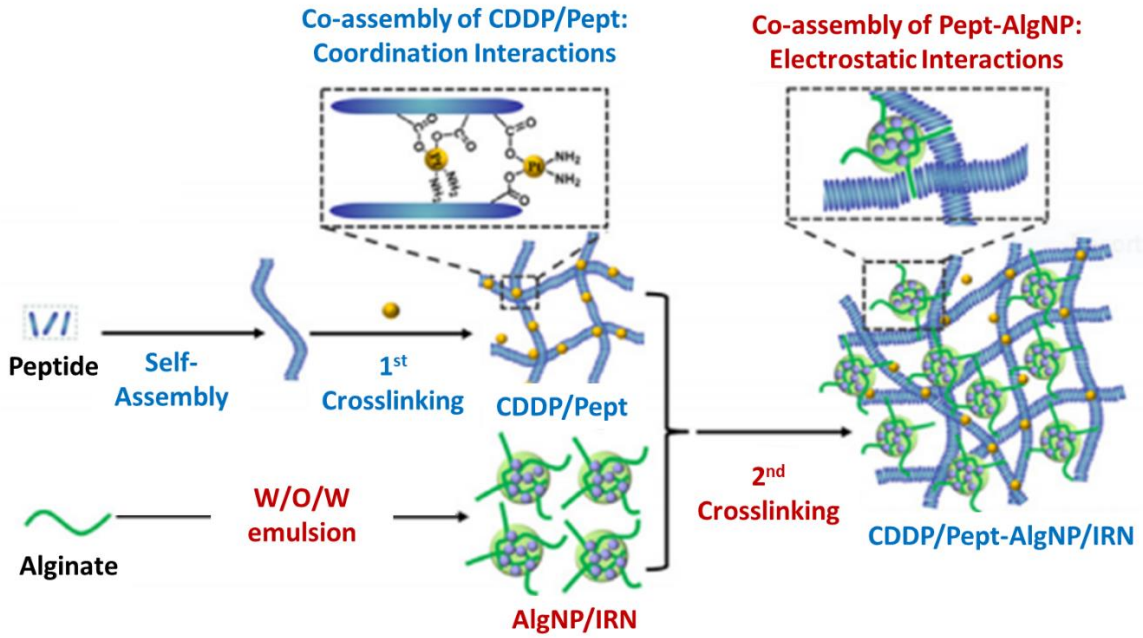

Figure 9: Schematic representation of nanocomposite hydrogel produced via double-crosslinking methodology for differential drug release in combination therapy [94]. Pept refers to a peptide whose sequence consisted of 2-Naphthylacetic acid-Phe-Phe-Tyr-Glu-Arg-Gly-Asp. Non-covalent coordination bonds were formed between cisplatin (CDDP) and Pept, which served as the hydrogel matrix. Irinotecan-loaded alginate nanoparticles (AlgNP/IRN) were prepared *via* water-in-oil-in-water (w/o/w) emulsion. AlgNP/IRN incorporation into the hydrogel matrix was possible through electrostatic interactions. The figures were adopted with permission from Wu, C.; Liu, J.; Zhai, Z.; Yang, L.; Tang, X.; Zhao, L.; Xu, K.; Zhong, W. Double-crosslinked nanocomposite hydrogels for temporal control of drug dosing in combination therapy.

In addition, Ibrahim et al. incorporated within alginate matrix both tamoxifen and silver nanoparticles [95,96], whose anticancer activity against colon tumors had been previously confirmed [97]. *In vitro* studies on breast cancer MCF-7 cells evidenced an enhanced anticancer effect, which was associated to an intensified production of reactive oxygen species (ROS), the down regulation of survival oncogenic genes, and cellular cycle arrest in G2/M phase. Furthermore, folic acid conjugation in particles' surface significantly improved the composite accumulation within the breast tumor cells.

Combination therapy can also involve, apart from chemotherapy, thermotherapy and radiotherapy. For instance, Alamzadeh et al. prepared an alginate hydrogel co-loaded with cisplatin and gold nanoparticles (AuNPs) [98]. AuNPs transform laser irradiation energy into heat and additionally, they make tumor cells more sensitive to radiation. The system was tested on human mouth epidermal carcinoma KB cells and resulted in the synergistic effect of chemotherapy, photothermal therapy and radiotherapy. Anticancer effects were enhanced and it would enable the decrease of drug and radiation dose.

3.2.1.1 Alginate-based hydrogels for oral drug delivery and colorectal cancer therapy

Several trials aim at the development of alginate-based oral delivery systems. Alginate bioadhesiveness and mucoadhesiveness, as well as its proved biocompatibility, are especially valuable properties for adhesive tablets formula. These can be easily introduced within the organism through a minimal invasive manner.

Alginate mucoadhesiveness is due to the interaction with glycoproteins of the gastrointestinal (GI) tract, what prolongs GI residence time and drug release. As a result, the bioavailability of encapsulated factors and the mechanism of action enhance [43]. In addition, alginates have been categorized by the U.S. Food and Drug Administration (US-FDA) as "Generally Referred

As Safe" (GRAS) material. This characterization allows an easier translation of innovative outcomes from bench to bedside [43].

Moreover, the ability of alginate beads to swell or shrink in response to pH makes them a potentially successful oral delivery vehicle. In the stomach, at low pH values, beads shrink and protect the encapsulated compounds. Across the intestine, pH increases and beads swell. Beads' surface porosity rises [47] and beads destabilize. Hence, loaded compounds are released [6,43,68].

For example, Bautista et al. developed an oral delivery system based on zein-alginate complexes for the encapsulation of limonin and nomilin [99]. Zein was known to provide beads with heat and water resistance and hence, to improve encapsulated compound's bioavailability. Beads' stability was proved *in vitro* with simulated gastric fluids. Encapsulated compounds' release was performed in simulated intestinal environment. In addition, both limonin and nomilin kept their chemopreventive bioactivities, namely free radical-scavenging and anti-angiogenic activity. Besides, Kiaei et al. prepared an alginate-pectin biocomposite for the encapsulation of folic acid [100]. Pectin was added so as to improve beads' stability in gastric environment. Also burst release of folic acid in stimulated GI fluids was prevented. Both approaches evidenced the potential of alginate as oral drug vehicle. For these reasons, alginate-based hydrogels are notably useful for the treatment of GI diseases though oral drug administration.

The International Agency for Research on Cancer places colorectum cancer in the top 3 of the most common types of tumor. Only in 2018 it supposed the 10.2 % of the new cases diagnosed to females and the 10.9 % diagnosed to males [1]. According to Global Cancer Statistics data, in 2018 the 9.2 % of cancer deaths were related to colorectal cancer and 881000 people died because of it [3]. In order to prevent colorectum carcinogenesis, Wang et al. prepared an oral colon-targeted delivery system composed of alginate and chitosan [56]. Both natural polymers where chosen for their mucoadhesive properties. Beads were loaded with icariin, whose protective and anti-inflammatory functions are well established. Studies in rats with induced colonic mucosal injury showed an increased colonic residence time and icariin release in the colon. Mucosal damage was reduced and hence, colon-protective effects were achieved.

As a way to develop an efficient colorectal cancer treatment, Sarangi et al. encapsulated naproxen within a matrix of alginate and Assam Bora rice starch [101]. Beads were extra coated with Eudragit S100, an enteric polymer known to improve colon targeting. Indeed, all *in vitro*, *ex vivo* and *in vivo* studies proved the colon-targeted effects of the oral delivery system.

Alginate functionalization has been broadly exploited for targeted delivery. It involves the inclusion of ligands within the alginate matrix so as to enhance beads' therapeutic effect [44]. For instance, Raza et al. incorporated chondroitin sulphate in 5-FU-loaded sodium alginate beads for selective colon delivery [102]. Chondroitin sulphate is commonly found in biological tissues and its accumulation within tumor stroma has been proved. *In vitro* characterization studies pointed this approach as a potential colonic targeted 5-FU vehicle when orally administered.

Folate has been widely employed for alginate functionalization. The reason is because different types of cancer cells over-express folate receptors. Not only enables folate targeted receptor-mediated endocytosis but also it promotes ligand-activated drug delivery and cellular accumulation [103]. With this aim, Rajpoot et al. attached folic acid (FA) to solid lipid nanoparticles (SLNs) loaded with irinotecan hydrochloride trihydrate (IHT) [104]. The composite was coated with alginate and an additional Eudragit S100 layer (Figure 10). The effectivity of the oral delivery system was demonstrated by *in vitro* cytotoxicity studies on COLO205 cells as well as on HT29 cells xenografted mice, both human colon cancer cell lines. Enhanced targeted anticancer effects for colorectal cancer treatment were evidenced.

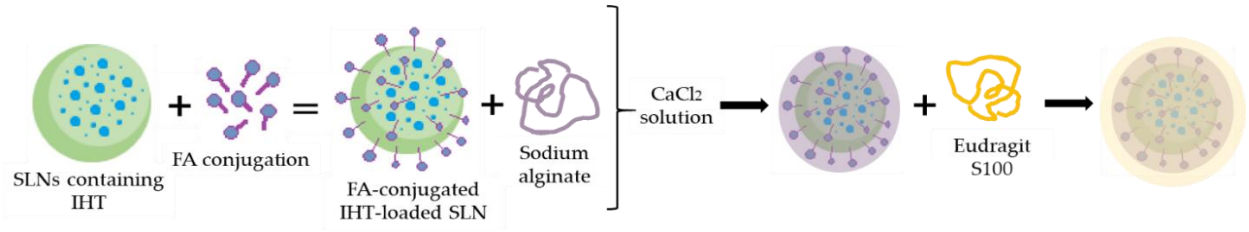

Figure 10: Schematic preparation of folic acid (FA)-grafted solid lipid nanoparticles (SLNs) bearing irinotecan hydrochloride trihydrate (IHT) and encapsulated in alginate matrix coated with Eudragit S100.

3.2.2 Alginate-based hydrogels as protein delivery systems

Alginate has been also employed as a vehicle for therapeutic proteins delivery. Given that ionotropic gelation is performed under mild conditions, adverse effects on protein structure and denaturalization are minimum [66]. In 2016, Sudareva et al. encapsulated superoxide dismutase (SOD), a therapeutic antioxidant enzyme [105]. The delivery system consisted of SOD-loaded calcium carbonate cores coated with alginate and gelatin A. In order to avoid protein degradation after oral administration, the trypsin inhibitors ovomucoid and soybean inhibitor were included. The oral delivery system proved to protect SOD activity in simulated intestinal fluids in presence of trypsin.

Similarly, Mahidhara et al. encapsulated iron-saturated bovine lactoferrin (isLF)–loaded calcium phosphate cores within a multilayer shell of chitosan and alginate [106]. Iron saturation have been pointed to enhance LF anticancer action. *In vitro* assays with breast cancer MDA-MB-231 cells demonstrated cellular uptake and anticancer effects. In addition, effective antitumor activity was confirmed after oral administration to MDA-MB-231 cells-bearing mice, a breast cancer xenograft model. What's more, none of the mice fed with isLF beads suffered from breast tumor recurrence whereas not fed mice did.

3.2.3 Alginate-based hydrogels as nucleic acid delivery systems

Gene therapy involves the release of genetic information. Hence, nucleic acid-loaded alginate beads may transfect adjacent cells so as to promote therapeutic proteins synthesis or to block cancer-related genes [63]. For example, Goldshtein et al. prepared a plasmid DNA (pDNA) carrier through the complexation of nucleic acids with alginate sulphate in presence of calcium ions [107]. The pDNA encoded for Diphtheria Toxin Fragment A (DT-A), which has intracellular toxic effects. It inhibits the synthesis of proteins, what results in cell death. Therefore, it is an appropriate reporter gene of cellular uptake. The efficiency of pDNA-loaded alginate beads for cancer gene therapy was proved in breast cancer MDA-MB-231 cells.

Besides, Rostami et al. encapsulated small interfering RNA (siRNA) for the dual inhibition of S1PR1 (S1P/sphingosine-1-phosphate receptor 1) and GP130 (glycoprotein 130) synthesis [108]. They aimed to stop cancer progression. siRNA was confined within an alginate-conjugated trimethyl chitosan matrix. The transfection efficiency was tested in different cancer cell lines: breast cancer 4T1 cells, melanoma B16-F10, and colon cancer CT26 cells. Cellular uptake and the blockage of cancer progression signaling pathways were demonstrated. Processes such as cell proliferation, angiogenesis, tumor cells survival and metastasis were effectively suppressed.

3.2.4 Alginate-based hydrogels as cell delivery systems

Alginate is the most employed biopolymer for cell encapsulation [42,45]. It acts as a semipermeable membrane that allows bidirectional transport of molecules. Nutrients and oxygen are allowed in whereas cellular waste is removed from the cellular environment [50]. Given the high surface / volume ratio, nutrient supply and gas exchange are enhanced [45].

Once introduced within an organism, encapsulated cells provide continuous synthesis of active compounds [11]. Not only enables cell therapy longer and sustained supply of therapeutic compounds but also, more complex release profiles [109]. Therapeutic agents such as small molecules and proteins are able to diffuse through the alginate layer [110]. Nevertheless, antibodies passage is impeded [109]. Alginate keeps encapsulated cells immunoisolation and

reduces the risk of rejection. As a result, co-administration of immunosuppressive therapies might be unnecessary [111]. Any negative effect has not been reported after alginate hydrogels transplant. These do not need to be removed from the host's organism because they are biodegradable and biocompatible [11].

Alginate hydrogels produced through ionotropic gelation offer further advantages. Hydrogel manufacturing under mild conditions preserves cellular integrity and viability. It guarantees cellular functionality and therapeutic factor's delivery. Moreover, fast alginate gelation reduces the time of cell manipulation.

Biological and mechanical features as well as the high-water content of alginate hydrogels provide cells with a suitable niche, similar to the natural extracellular matrix. In addition, physico-chemical properties can be tailored for specific cell types requirements [45].

For instance, Saenz del Burgo et al. encapsulated genetically modified human HEK-293 cells within alginate-poly-L-lysine matrix [112]. Cultured cells secreted recombinant bispecific antibodies (bsAbs): anti-CEA (carcinoembryonic antigen) x anti-CD3. These bsAbs bound tumor-associated CEA and simultaneously promoted peripheral blood lymphocytes activation. Results evidenced the potential of this approach to eradicate CEA expressing tumor cells through local T-cell activation.

In regards to *in vivo* studies, Johansson et al. encapsulated genetically modified human glioblastoma BHK cells within alginate [113]. Cells overexpressed the extracellular part of protein Lrig1 (sLrig1), which is a tumor suppressor that induces EGFR downregulation and degradation. EGFR gene is commonly amplified and mutated in glioblastoma cancer cells. Therefore, the obtained beads were introduced into the brain of glioma-bearing mice. Results demonstrated Lrig1 inhibition of tumor growth and the improvement of mice survival.

A different approach involved the encapsulation of bacterial cells. Funaro et al. used alginate to immobilize recombinant *E. coli* [114]. Cells were genetically modified and synthetized cytosine deaminase (CD), which catalyzes the conversion of 5-fluorocytosine, a non-toxic prodrug, to 5-FU. *In vitro* assays with rat 9L glioma cells proved an anticancer effect similar to that of free 5-FU. However, this strategy would allow a localized 5-FU action and hence, systemic toxicity could be avoided.

3.3 Alginate-based hydrogels as cell culture substrate

Physico-chemical characteristics of alginate hydrogels make them similar to the extracellular matrices of soft biological tissues [42,45]. In addition, they hold a high-water content. Because of the similarities with macromolecular-based biostructures, alginate hydrogels are employed as scaffolds for cell culture and they are useful for *in vitro* studies and assays [42].

For instance, Mandal et al. made use of sodium alginate to study the influence of Wharton's jelly-derived mesenchymal stem cells (WJMSCs) on breast cancer stem cells (CSCs), namely MDA-MB-231 and MCF-7 cell lines [115]. Therefore, WJMSCs were encapsulated and evaluated. Findings in gene expression and paracrine profile considerably differed whether cells were seeded in a 2-D monolayer culture or in an alginate 3-D scaffold. In the 3-D hydrogels WJMSCs stem cell properties remained and their therapeutic effect was enhanced. Alginate hydrogels enabled a more realistic evaluation of cellular interactions in the natural tumor environment. Results determined the antitumorigenic effect of WJMSCs on breast CSCs.

In addition, Kletzmayr et al. seeded ovarian cancer SKOV3 cells, lung cancer A549 cells and prostate cancer LNCaP cells in an alginate-chitosan 3-D culture. Cells were exposed to doxorubicin and paclitaxel in order to identify possible chemoresistance-associated therapeutic targets [116]. After a proteomic screening, tumor-associated antigens were recognized and targeting antibodies were produced. Hydrogels allowed a feasible assessment of the immunotherapy effectiveness. Moreover, a more automated procedure in regards to cell seeding, media and therapeutic agents supply, and cell viability test could be performed.

Otherwise, El-Sayed et al. enclosed *Aspergillus Fumigatus* and *Alternaria Tenuissima* in calcium-alginate capsules for the production of Taxol [117]. Taxol is a therapeutic compound broadly applied for cancer chemotherapy and other diseases. It is obtained from some vegetal *Taxus* species and thus, its application is not sustainable nor eco-friendly. Both fungal strains

were capable to produce Taxol. Furthermore, the encapsulation conditions were adapted to optimize Taxol productivity.

3.4 Further applications of alginate-based hydrogels

Alginate hydrogels have been combined with other compounds such as metals and carbon-based nanomaterials to ameliorate immunosensing devices. Alginate functionalization with antigens or antibodies is performed to enable signal transduction and tumor biomarkers detection. Such is the example of Zhao et al. who prepared a sodium alginate-based immunosensor for the detection of prostate-specific antigen (PSA) [118]. The design was a sandwich-type immunoassay based on electrochemical signal transduction. The device was proved to be specific and stable. It was sensible enough to determine up to 0.9 fg/ml of PSA.

Besides, Lee et al. developed a system to improve laparoscopic operations based on alginate hydrogels loaded with human serum albumin (HAS) and indocyanine green (ICG), a dye commonly applied for imaging diagnostic test [119]. The effectivity of the hydrogel as fluorescent surgical marker was tested in a porcine model. It was injected into submucosal spaces of the porcine stomach the fluorescent signal and persisted up to 3 days after without diffusing. Therefore, this laparoscopic fluorescence imaging system was proved as a potential strategy to accurately define tumor location during surgery.

Conclusion

In summary, this review provides an overview of alginate properties, chemical structure and hydrogel formation ability by means of ionotropic gelation. Given its suitability for a broad spectrum of biomedical applications, it can be said that alginate has good prospects in cancer therapy and research. As we could see throughout this review, not only is it a smart vehicle for anticancer drugs, proteins, nucleic acids and cell delivery but also an efficient 3-D scaffold for both cell culture and immunosensing, and fluorescent surgical marker.

Given its ability to shrink, the mucoadhesivess as well as the GRAS classification by the US-FDA, alginate is a successful component of oral delivery systems, particularly valuable for the treatment of GI tumors. In addition, the possibility of alginate functionalization and the combination with other polymers allows a more accurate delivery of therapeutic compounds at targeted parts of the organism.

Despite the fact that clinical application of alginate-based hydrogels is still limited, it is a promising biomaterial for cancer treatment and research. Alginate is expected to improve traditional therapies and overcome current associated drawbacks such as tumor resistance and systemic toxicity. All in all, alginate hydrogels promote new encouraging opportunities for cancer patients' total recovery.

Supplementary Materials:

Author Contributions: Writing—original draft preparation, Belen Reig-Vano; writing—review and editing, Bartosz Tylkowski, Xaviar Montané and Marta Giamberini. All authors have read and agreed to the published version of the manuscript.

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