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How Are Natural-Based Polymers Shaping the Future of Cancer Immunotherapy—A Review

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ABSTRACT

With the increasing knowledge of cancer pathophysiology, new therapeutics based on the modulation of the immune system have been developed, overcoming many of the disadvantages of traditional pharmaceuticals. Several immunotherapy systems have in fact become the preferred treatments to tackle particular types of cancer. Despite these impressive clinical results, issues, such as biomolecule susceptibility to proteolytic degradation and tumor microenvironment immunosuppression need to be overcome to further increase efficacy and safety upon use in patients. Natural-based polymers have shown the potential to address some of these limitations. Widely used in the field of tissue engineering and regenerative medicine, these polymers have been increasingly incorporated in the development of improved immunotherapeutics due to intrinsic properties, such as biocompatibility and bio-similarity. In this review, the novelties these polymers have brought to immunotherapy and their implementation to create new and more complex therapeutics are outlined, and emerging trends are identified. We argue that to fully exploit the potential of natural-based polymers, improved interaction between clinicians and material scientists must come to the fore. Concurrently, material scientists must intensify efforts to overcome the problem of batch-to-batch variability in natural-based polymers to streamline clinical application. All-in-all, we envision a bright future for natural-based polymers in immunotherapy.

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Immunotherapy; natural-based polymers; cancer; immunomodulation; antigen presentation

1. Introduction

The impact of the tumor's microenvironment on the efficacy of current cancer therapeutics has become the object of increased study.[1–3] It has been established that tumor extracellular matrix, intratumoral hypoxia, tumor resident cells, and the immunosuppressive capacity of cancer cells may hamper currently applied treatments. Dendritic as

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well as T-cells, responsible for conducting anti-tumoral responses, are some of the most affected cell types[4,5] and this immunosuppression leads to tumor cell expansion and migration. This immunosuppressive capacity has led to the development of the concept of "Immunotherapy" which was initially introduced as a form of biological therapy to boost the natural immune response and to aid the host in responding against a certain pathology. This was demonstrated early on by blocking the immunosuppressive effects of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) with antibodies which in turn resulted in the rejection of tumors in immunocompetent mice models.[6] Since then, it has been widely adopted in many forms to stop or slow tumor growth and avoid tumor metastasis. Several different methods of immunotherapy have been developed namely immune checkpoint inhibitors, adoptive T-cell therapy (ACT), monoclonal antibodies, artificial antigen presenting systems, and immunological adjuvants.

While these systems have made a name for themselves, the introduction of biomaterials in this field has allowed the development of novel systems comprehending greater biological similarity toward native molecules. This plays greatly in favor of increasing the biocompatibility of these systems while additionally bringing new properties to the table. Natural polymers are among the most promising biomaterials. They are naturally occurring polymers readily available in nature or extracted from plants or animals. Some examples of these natural occurring polymers are proteins, carbohydrates, or even nucleic acids. Many of these natural materials are known for their outstanding biological performance, such as tensile strength, adhesive properties, superhydrophobicity, toughness, self-healing, and self-assembly.[7–10] The application of biomaterials in the field of cancer immunotherapy has to some extent overlapped requirements with the field of tissue engineering and regenerative medicine. This has helped fuel the development of novel immunotherapeutics based on natural materials. Examples of these are spherical micro or nanoparticles, nano, and microcapsules, micelles, artificial antigen presenting systems, hydrogels, microneedles, scaffolds, and natural based immunoadjuvants. These systems have been applied in a standalone manner or in combination with more conventional therapies, such as chemo- and radiotherapy.

Throughout this review, we will address the main advantages of using natural based systems when compared to more conventional synthetic polymers, how these systems may play an important role in overcoming certain key issues in immunotherapy, and how they may aid in shaping the future of immunotherapy.

2. Evolution of therapeutics in cancer immunotherapy

Immunotherapy may be defined as a form of treatment that utilizes the immune system, either by activation or suppression, to overcome an illness. This may encompass autoimmune diseases, hypersensitivities, and different forms of cancer. Immunotherapeutics are considered a form of biological therapy, applied to direct immune responses. Some of the tools used to achieve this goal include the administration of immunomodulating agents, such as interferons, interleukins, colony-stimulating factors, monoclonal antibodies, and different forms of vaccines.[11–13] With the field focused on creating new more precise and effective systems, several developments have been made over the past years which can be grouped into two main categories: artificial antigen-presenting systems

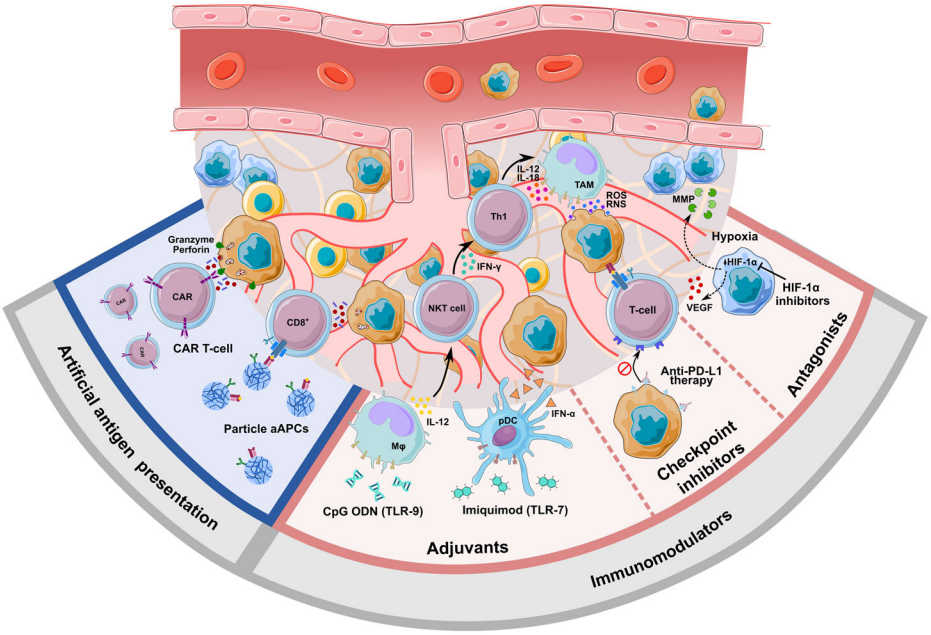

Figure 1. Immunotherapy strategies for the treatment of cancer. There are 2 main approaches used for cancer immunotherapy: (1) artificial antigen presentation (blue); (2) immunomodulators primarily consisting of adjuvants (red—left), checkpoint inhibitors (red—center), and antagonists (red—right).

and immunomodulators (Fig. 1). While the first can lead to specific immune responses toward a desired epitope or tumor associated antigen (TAA), the later focuses on molecules capable of regulating immune responses in either an immunostimulatory or immunosuppressive manner. Some commonly known types of immunomodulators are checkpoint inhibitors, cytokines, agonists, and adjuvants. These two build on each other as they may be used in a combined form to potentiate a desired response or pathway.

2.1. Artificial antigen presentation

Antigen presentation is the key mechanism of action by which the immune system develops antigen-specific responses. This mechanism of action is dependent on the presentation of proteins to lymphocytes in the form of short peptide fragments or antigens by means of professional antigen-presenting cells (APCs).[14] Protein antigens are broken down into peptides and presented in conjunction with either class I or class II major histocompatibility complex (MHC) molecules on the cell surface which will then interact with the appropriate T-cell receptor to generate an effective immune response. Additionally, complementary signaling is required, such as the cluster of differentiation (CD)28-CD80/86 interaction, followed by cytokine production.

The understanding of how antigens are presented to immune cells and the ability of the tumor microenvironment to suppress anti-tumor responses has led to the conceptualization of artificial antigen presentation. This may be performed by one of two main strategies (i) an indirect approach which consists in delivering a specific antigen to circulating antigen-presenting cells, for it to be presented on their surface by MHC

molecules, and (ii) a direct method in which a delivery system is functionalized with co-stimulatory molecules, such as antigen-loaded MHCI or MHCII molecules and anti-CD28 antibodies for a direct interaction with host T-cells. It was based on the latter that the concept of "artificial antigen-presenting cells (aAPCs)" was created. The strategies used to develop these artificial antigen-presenting systems can be of cellular or acellular nature.

Cell-based strategies may be based on various cell sources from autologous to xenogenic and even exosomal strategies. ACT is one of the most common immunotherapy strategies and consists of the *in vitro* expansion of tumor antigen-specific T-cells which are then infused back into their patients.[15,16] One of the greatest handicaps of this system is the lack of efficiency in terms of generating a great number of antitumor T-cells in a short period of time.[17,18] This not only renders the technique costly but also requires an exhaustive process before clinical application. The fate of ACT in terms of clinical efficacy is largely dependent on how the antigen-specific T-cells are stimulated during the priming phase[19–21] and dependent on downstream stimulation with γ chain receptor cytokines, such as interleukin (IL)-2, IL-7, IL-15, and IL-21.[22–24] An inefficient priming may result in hyporesponsive or anergic T-cells[25] resulting in a lack of proliferation and/or loss of effector function when in contact with the respective antigen, rendering the treatment ineffective. However, on the contrary, excessive stimulation of the cells *in vitro* may also compromise treatment efficiency due to T-cell induced cell death (AICD).[26,27] Therefore, a fine balance is required to obtain a high number of effector T-cells and this is where particle-based methods gain a clear advantage as these are highly tunable systems with controllable properties. Alternative cell-based methods, such as gene-engineered K562 cells,[28] have been developed to overcome some of these issues, as they lack the expression of endogenous human leukocyte antigens (HLA) class I, II or CD1d, as well as of co-stimulatory molecules, such as CD86, CD83, TNF superfamily member 4 (TNFSF4), inducible T cell costimulator ligand (ICOSL) or CD40L.[29] Additionally, their lack of expression of inhibitory molecules like programmed death-ligand 1 (PD-L1), PD-L2, B7 homolog 3 protein (B7H3), and B7H4,[29] contributes to a continued effector function even in an immune suppressive microenvironment. Tumor-specific T-cell expansion, through the use of autologous dendritic cells derived from a patient's peripheral blood mononuclear cells (PBMCs), is another popular method to induce tumor-specific responses. Herein, immature dendritic cells (DCs) are activated and matured by stimulation with specific factors, such as polarizing cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4,[30] while additional downstream re-stimulations are required to generate enough tumor-infiltrating lymphocytes (TIL). However, inconsistent generation of effector memory T-cells (T_{EM}) regarding function or persistence[31] has been a limiting factor. Another alternative strategy has been the creation of chimeric antigen receptor (CAR) T-cells, defined as genetically modified T-cells that encode for transmembrane chimeric molecules with dual functions: (a) immune recognition of tumor antigens expressed on the surface of tumor cells (b) active promotion and propagation of signaling events controlling the activation of the lytic machinery.[32] Treatment efficacy is highly dependent on the: (i) choice of the target epitope; (ii) architecture of the produced CAR; (iii) method of administration ranging from doses to frequency; (iv) efficient tumor homing and survival in the tumor

microenvironment; (v) patient lymphocyte depletion before administration of CAR-T-cells.[33] Yet, while promising, this system is not without its limitations namely, the lack of specific targetable antigens and inefficient trafficking toward the tumor foci due to the unbalanced secretion of cytokines from tumor cells, such as chemokine (C-X-C motif) ligand 9 (CXCL9) and CXCL10.[33] Additionally, the tumor microenvironment also presents a series of obstacles related to inhospitability and inaccessibility to immune cells due to hypoxia, low nutrients and ultimately the high concentration of acidic metabolites that hamper T-cell proliferation and cytokine production.[33]

On the other side of the spectrum lie particle-based aAPCS which have fueled the debate of whether size matters for antigen delivery.[34–37] While highly dependent on the final application of the system, studies have reported that macroparticles mimicking APC size tend to produce better results in terms of achieving stable, tight synapse-like contacts with the ligand-displaying microparticles. Conversely, nanoparticles offer other advantages, such as surface area to volume ratio, the capacity to deliver therapeutic cargo, and the ease in crossing various anatomical niches, while having been reported to induce antigen-specific T-cell proliferation *in vitro* and lead to effective T-cell stimulation and inhibition of tumor growth *in vivo*.[38] Together with size, particle shape variance has also been a characteristic known to promote aAPCs/T-cell interactions.[39–41] However, despite size, shape, and core material differences, all of them share common properties, such as high tunability and reproducibility in stimulating T-cells across batches, which makes them ideal candidates for the preparation of aAPCs when compared to cell-based methods, which display numerous variability issues.

2.2. Immunomodulators

Immune adjuvants, also known as immunopotentiating agents, are a subset of agents with unique properties capable of increasing, improving, or extending immune response against antigens administered simultaneously, hence improving the immunogenicity of antigens by decreasing the amount and number of immunizations. Immune adjuvants have been around for decades with early versions consisting of aluminum precipitates.[42] While the field has evolved immensely, aluminum salts continue to be widely used as adjuvants with different chemical variations being developed commercially over time, such as Alhydrogel® and Adju-Phos®, intended for human use. Other adjuvants have come into play over the years, such as oil-water emulsion adjuvants (Freund's Adjuvant and Squalene), adjuvants of bacterial origin Flagellin, bacterial membranes, Monophosphoryl Lipid A (MPL-A), Muramyl dipeptide and adjuvants from bacterial DNA (CpG oligodeoxynucleotides). All of these systems present specific ways of interaction with the immune system to potentiate antigen responses. Aluminum adjuvants are known to form precipitates that promote phagocytosis of antigens by antigen-presenting cells, as well as to induce local inflammation *via* the NLRP3 inflammasome. Upon activation of this pathway, the secretion of mature IL-1β and IL-18 by dendritic cells and the differentiation of T helper 2 (Th2) cells are triggered, promoting the activation of B cells and the subsequent production of antibodies.[43] Oil-water emulsions, such as the case of Freund's Adjuvants, contain inactivated mycobacteria which in turn attract macrophages and other cells to the site of injection. Other oil-water based adjuvants, such as GLA-SE

are known to induce strong signaling through the toll-like receptor (TLR)-4, caspase, IL-18, and interferon (IFN)-γ pathways, leading to a T helper type 1 (Th1) response.[44] Regarding bacterial adjuvants, two have stood out, MPL-A and CpG oligodeoxynucleotides. The former consists of structurally modified lipid A, a component of lipopolysaccharide (LPS), which has been known to induce the maturation of DC cells, $CD4^+$ T-cell clonal expansion, and Th1 responses without the inflammatory effects of LPS.[45] Regarding CpG oligodeoxynucleotides, these are synthetic oligodeoxynucleotides (ODN) that contain unmethylated CpG motifs (CpG ODN). When these CpG motifs are unmethylated they induce macrophages to secrete IL-12, which induces IFN-γ secretion by natural killer (NK) cells and therefore may induce Th1 differentiation.[46]

Several hallmarks of cancer are known to regulate the tumor microenvironment, promoting tumor growth and survival. Two of the most studied are the capacity to escape immune destruction and the tumor's ability to promote angiogenesis.[47] The balance between the capacity of one's immune system to control and abolish tumor growth and the inherent ability of the tumor to evade the immune system dictates tumor aggressiveness. An imbalance in these mechanisms is known as immunoediting.[48] Two key molecules expressed on activated T-cells and known as inhibitory T-cell checkpoints, CTLA-4 and programmed cell death protein 1 (PD-1), are behind this mechanism. Several strategies have been developed to interrupt this immune suppressive capacity of tumor cells, namely through the development of checkpoint inhibitors, such as antibodies with blocking potential. In the case of CTLA-4 binding, Ipilimumab has been developed for the deactivation of the inhibitory signals toward T-cells.[49] Regarding the targeting of PD-1 and of PD-L1, antibodies, such as nivolumab, pembrolizumab, and pidilizumab have been created to block tumor T-cell interactions.[49]

On the other hand, tumor survival is in a big part sustained by neo-vascularization, in which tumor hypoxia has been shown to play an important role. In tumors, hyperproliferating cancer cells overgrow their blood supply and become hypoxic. This hypoxic microenvironment in turn creates an imbalance between angiogenic activators and inhibitors[50] leading to rapid and chaotic blood vessel formation which tends to be of abnormal character, underdeveloped and leaky. This continuous flow of nutrients and oxygen ensures the continued tumor cell proliferation and angiogenesis. Several key players, such as hypoxia-inducible factor 1-aplha (HIF-1α), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, mitogen-activated protein kinases (MAPK), nuclear factor kappa B (NFκB) and equally vascular endothelial growth factor (VEGF)-A[51–53] have been tied to tumor progression and poor prognosis. With this knowledge, angiogenic inhibitors have been introduced, for instance, CRLX101 for HIF-1α inhibition (Clinicaltrials.gov Identifier: NCT01652079), SU5416 (Semaxanib®) for the selective inhibition of VEGF receptor (Flk-1/KDR) through the decrease of HIF-1α protein and VEGF mRNA *via* the PI3K/Akt pathway (ClinicalTrials.gov Identifier: NCT00005642), mTOR inhibitor Rapamycin®, known to inhibit both the stabilization and the transcriptional activity of HIF-1α in hypoxic cancer cells[54] or monoclonal anti-VEGF-A antibodies, such as bevacizumab (Avastin®).[55]

While these different strategies have shown clinical efficacy, they are not free of immune-related adverse events. Checkpoint inhibition has been shown to lead to impaired self-tolerance resulting from the loss of T-cell inhibition, while much broader

complications, such as gastrointestinal, hepatic, and endocrine toxicities are many times visible in patients. Combinatorial therapy is a promising way to overcome these issues. By combining different therapeutic strategies, dosing may be regulated to sub-toxic levels potentiating the effect of these therapeutics. Another strategy that has gained traction over the years is targeted delivery. By delivering lower doses of these drugs to the region of interest, the tumor microenvironment in the case of cancer, a localized effect may be seen without systemic toxicities being achieved. Different systems have been developed over the years to accurately deliver these agents, from polymeric to metallic nano or micro-particles with varying shapes and structures.

3. Basics of natural polymers

Polymers are chemical compounds made up of small molecules otherwise known as monomers, arranged in simple repeating structures bonded chemically through covalent bonds to form a large molecule or chain.[56] These compounds can be generally of three main sources, synthetic, semi-synthetic, or of natural origin. The latter occurs in nature and can be extracted from plants, animals, and even bacteria. Natural polymers are crucial for human existence as these encompass proteins and nucleic acids, cellulose, natural rubber, starch, or even honey and wool. The advantages and disadvantages of natural *vs.* synthetic polymers in several fields have been a recent topic of interest.[57–60] While structure controllability and reproducibility are two important features of synthetic polymers, the bio-similarity, biodegradability, biocompatibility, and environmental friendliness of natural polymers are very appealing properties to the biomedical community. Several natural-based polymers have been adopted in the biomedical field, namely in tissue engineering and regenerative medicine, being also selected in the design of novel immunotherapeutics.

3.1. Chitin/chitosan

Chitin is a marine-origin natural polymer and can be found in a wide variety of arthropod shells, being collected essentially from crustaceans. Structure-wise, it resembles cellulose, being a linear, high molecular weight crystalline polysaccharide composed of N-acetylated glucosamine (2-acetylamino-2deoxy-D-glucopyranose) units linked by β-(1 → 4) glycosidic bonds[61] which contribute to its strength. Chitosan is a popular chitin derivative due to features, such as hydrophilicity and ready solubility in dilute acids. It can be obtained through the partial alkaline deacetylation of chitin and displays a semi-crystalline cationic structure, comprised of β-1,4-linked 2-amino-2-deoxy-D-glucose.[62] It proves insoluble in aqueous solutions with a pH higher than 7 and soluble in dilute acids (pH 6) due to its free amine groups which become protonated. What makes it an attracting natural polymer is its non-toxicity, biodegradability, biocompatibility, and antibacterial properties.[63]

3.2. Cellulose

Cellulose is a well-known natural polymer that is heavily used in the paper and textile industries. It is widespread and can easily be found in the cellular wall of plants, more specifically within the stems, stalks, or trunks.[64] Additionally to its presence in plants,

some microorganisms have been known to equally express this polymer, namely gram-negative bacterium *Acetobacter xylinum.*[65] Cellulose is composed of β-D-glucopyranose units linked by (1 → 4) glycosidic bonds which are formed through the polymerization of glucose residues from substrates, such as uridine diphosphate glucose (UDP)-glucose.[66] These glycosidic links possess a specific stereochemistry that creates linear glucan chains that enable precise and ordered interactions between different chains. Structurally, this polymer exists in the form of microfibrils, consisting of various chains strongly linked by hydrogen bonds conferring to this natural polymer a rigid structure,[67] consequently accounting for a high degree of crystallinity, low solubility, and poor degradation *in vivo*. However, features like its high strength in the wet state and its biocompatibility make it an appealing natural polymer.

3.3. Alginate

Alginate is an anionic natural polymer that can be found in brown algae.[68] Structurally, it comprises (1–4)-linked β-D-mannuronic acid and α-L-glucuronic acid units, organized in regions of sequential mannuronic acid units, guluronic acid units, or through a combination of both.[69] One of the key elements that make alginate such an interesting polymer for the biomedical field is its ease of gelification in the presence of divalent cations (Ca^{2+}, Mg^{2+}, or Ba^{2+}). Crosslinking occurs between the carboxylic groups present in the alginate backbone, therefore forming hydrogels.[70] Its well-known structure has allowed its chemical modification through the carboxylic groups present in the guluronic acid residues, such as in the case of functionalization with arginylglycylaspartic acid (RGD) peptides to increase cell-material interactions.[71] Additionally, its low toxicity has also contributed to its increased demand as an alternative to synthetic polymers.

3.4. Hyaluronic acid

Hyaluronic acid (HA), or hyaluronan, is a non-sulfated linear negatively charged polysaccharide that consists of alternating repeating units of the β-1,4-D-glucuronic acid–β-1,3-*N*-acetyl-D-glucosamine disaccharide.[72] It may originate from several sources, however, the most predominant are rooster combs and bacterial expression systems in *Streptococcus.*[73]

Variations in the source lead to different rheological properties. Attributes, such as its solubility in water and its shear-dependent viscosity, which allows for it to be injected through a small gauge needle, make this polymer interesting for biomedical applications.[74] Additionally, its amenability to enzymatic degradation[75] by HAase and papain contributes to its biodegradability which makes it compelling for *in vivo* applications.

HA is a main component of the extracellular matrix and promotes both cell motility and proliferation.[76] Two main forms of HA have been described,[77] a low-molecular weight HA which triggers proinflammatory responses, and a high-molecular weight HA which has been associated with anti-inflammatory cues. Its role comes through the interaction with several types of immune cells, endothelial cells, fibroblasts, and keratinocytes *via* cell surface receptors, such as receptor for HA-mediated motility (RHAMM), CD44s, TLR-4, TLR-2, Stabilin-1 (HARE), and lymphatic vessel endothelial hyaluronan receptor-

1 (LYVE-1).[78] The interaction of HA with its receptor CD44 which in turn is highly expressed in many cancers and is capable of regulating the metastatic process,[79,80] has allowed for the development of tumor-targeting systems as in the case of vaccines. Additionally, its ability to undergo enzymatic degradation makes it an enticing natural polymer for the development of several immunotherapeutic strategies. Since HA became a widely studied polymer over the years, modifications in its chemical structure were frequently introduced,[81] such as the formation of esters through the esterification if its carboxylic groups[82] which promotes resistance to a range of conditions and facilitates processing into membranes, spheres, particles and porous structures.

3.5. Gellan gum

Gellan gum (GG) or gellan is a quite common natural polymer used in the food industry. It is a linear anionic polysaccharide composed of tetrasaccharide repeating units of 1,3-β-D-glucose, 1,4-β-D-glucoronic acid, 1,4-β-D-glucose, 1,4-α-L-rhamnose, containing one carboxyl side group.[83] This polymer is known to exist under two forms, acetylated—which is its original form produced by the *Sphingomonas paucimobilis* bacterial strain—and the deacetylated—the most widely available and able to undergo processing.[84] Similarly, to others, it is capable of ionotropic gelation. Its gelation is strongly influenced by the chemical nature and amount of cations present in the solution, being promoted in a stronger manner in the presence of divalent cations when compared to monovalent cations.[85] Upon crosslinking through the presence of these divalent cations, the gelation occurs *via* chemical bonding between the cation and two carboxylate groups belonging to glucuronic acid molecules in the GG chain.[86] However, temperature shifts induce structural changes which account for the thermoreversible nature of the polymer.[87]

4. Novel developments in cancer immunotherapy driven by systems using natural polymers

The development of novel systems for the vaccination, artificial presentation of TAA's, or the delivery of immunomodulatory molecules has been proven challenging due to issues related to antigen or protein stability. When developing new platforms for delivery, it has to be considered how liable these peptides are to mechanical clearance and proteolytic degradation in the microenvironment, which may lead to antigen denaturation and loss of antigenicity.[88]

The use of natural polymers for the development of these systems has allowed not only to exploit properties, such as biocompatibility and biodegradability but also other key aspects, such as ionotropic gelation capability or their structural resemblance with human extracellular matrix proteins. This allowed room for the development of systems based on, *e.g.*, micro- and nano- particles and injectable hydrogels which can ensure the protection of key molecules from the hostile tumorigenic environment while potentiating immune-stimulatory effects, therefore increasing their half-life upon administration. Here, we will review some of the most recent systems developed in the field of immunotherapy involving the use of natural polymers (Fig. 2).

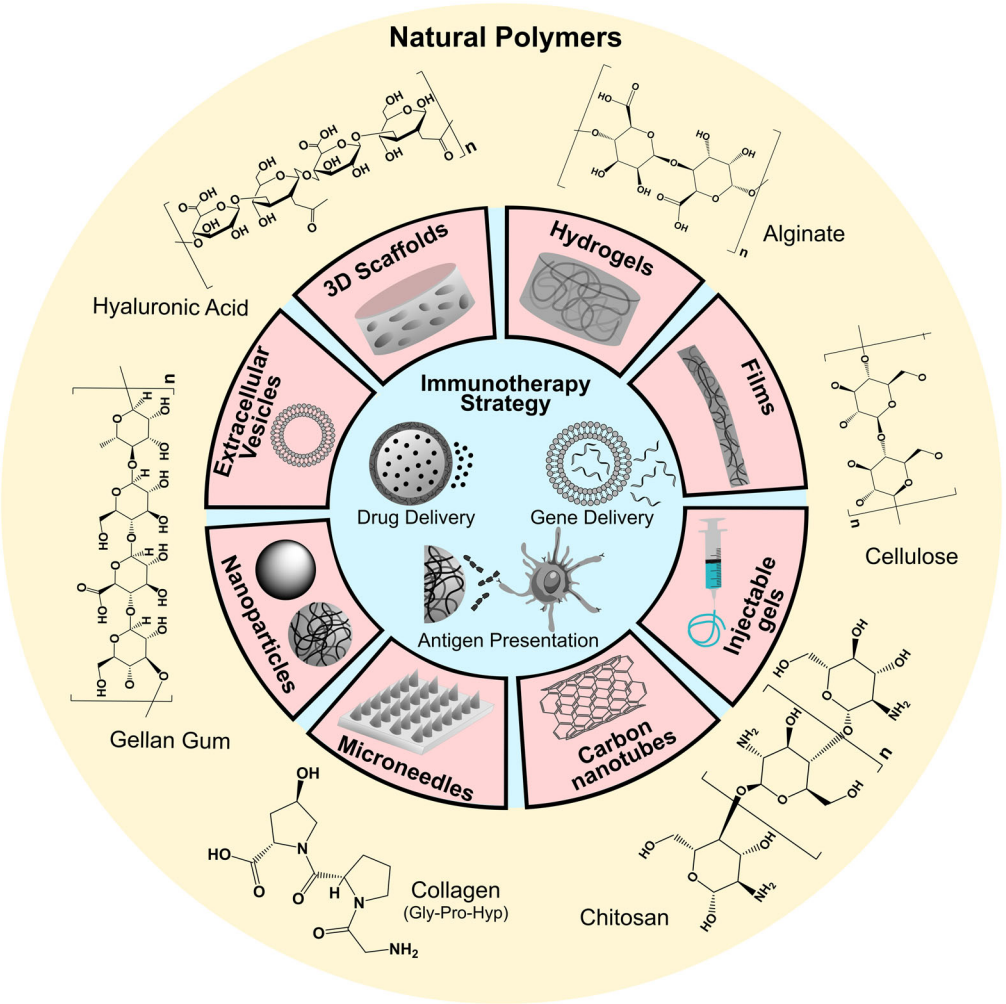

Figure 2. Natural polymer-based systems for the development of immunotherapeutics.

4.1. Gel-based systems

While the use of cross-linkable natural polymers in the field of tissue engineering and regenerative medicine (TERM) is widespread, this technology is fairly young in the development of immunotherapeutics. An *in situ* cross-linkable hydrogel for immunotherapeutic use has been described using alginate.[89] This polymer was used as a delivery system for catalase (Cat) and CpG oligonucleotides for cancer therapy (Cat/ALG). The rapid gelation of the system by intra-tumoral Ca^{2+} allowed for both a homogeneous distribution of ^{131}I-Cat throughout the tumor as well as a long-term entrapment of ^{131}I-Cat without leakage into the surrounding healthy tissues. Hence, the decomposition of the tumor endogenous hydrogen peroxide (H_2O_2) was triggered, contributing to higher intra-tumoral oxygenation values maintained over 72 hr. This originated greatly reduced levels of HIF-1α upon Cat/ALG treatment. When assessing the potential of the system in radioisotope therapy (RIT) using a 4T1 murine tumor model, ^{131}I-Cat/ALG resulted in tumor-free animals with prolonged survival without any deaths. A patient-derived

xenograft (PDX) mouse tumor model was also used to better represent real human patient tumors where the ^{131}I-Cat/ALG led to 100% elimination of PDX tumors. When tested in a larger animal model of rabbits bearing VX_2 tumors, a similar effect was seen, where ^{131}I-Cat/ALG injection resulted in complete tumor regression within 2 weeks and sustained survival for over 150 days. When CpG oligonucleotides were mixed with the ^{131}I-Cat/ALG system and used in combination with intravenous administration of a CTLA-4 antibody, suppression of distant tumor growth was seen. Additionally, the ratio of CTL/regulatory T-cells (T_{reg}) was significantly increased, resulting in increased TNF-α and IFN-γ secretion. Long-term immunological memory was confirmed through a higher number of T_{EM} ($CD3^+CD8^+CD62L^-CD44^+$) residing in both lymphoid and non-lymphoid tissues as well as protection when animals were rechallenged with secondary tumors. Other injectable hydrogel-based systems relying on alginate have also been reported for cancer immunotherapy as the case of the introduction of a persistent luminescence material (PLM) and an immunoadjuvant (R837) into alginate-Ca^{2+} gels[90] which allowed to develop a system that would allow to amplify the immunogenicity of tumor-associated antigens originating from persistent luminescence sensitized photoimmunotherapy (PDT) therefore leading to a more robust immune response to suppress tumors *in vivo*. In another study, alginate was conjugated to a triphosphate (ATP)-specific aptamer hybridized with CPG to produce a hydrogel that would form *in situ*.[91] This smart hydrogel upon low doses of chemo-/radiotherapy would trigger the release of CpG. Verbeke and colleagues have also contributed to alginate-based injectable systems for the recruitment and activation of immune cells.[92,93] In a similar fashion, GG was used to develop an injectable hydrogel for combinatorial photothermal-immunotherapy of cancer.[94] GG co-loaded with Dawson-type polyoxometalate (POM) and Toll-like receptors agonist resiquimod (R848), exhibited high photothermal conversion efficiency eliciting a high tumor inhibition rate of 99.3% together with significantly elevated TNF-α, IL-2, and IL-6.

Nucleic acid-based vaccines have made their way into the field of immunotherapy due to their several advantages when compared to more traditional protein-based vaccines. This was clearly evidenced recently with the use of this technology to develop two of the first United States Food and Drug Administration (FDA) approved mRNA vaccines to deal with the COVID-19 pandemic, namely the vaccines developed by BioNTech[95] and Moderna.[96] The use of pDNA or mRNA-based vaccines allows the development of $CD8^+$ T-cell responses while not being subject to neutralization through immunosuppression, therefore allowing for repeated antigen challenging. Natural origin-based polymers can further contribute to develop more efficient, biocompatible, and biodegradable delivery systems for these vaccines. For both BioNTech and Moderna vaccines, lipid nanoparticles consisting of ionizable lipids were used for the safe and efficient delivery of mRNA encoding SARS-CoV-2 S(2P).[97,98] Injectable hydrogels have also aided the administration of DNA vaccines. Injectable N-succinyl chitosan (S-CS) with oxidized alginate (O-Alg) gel scaffolds have been applied for the local delivery of ovalbumin (OVA) mRNA lipoplexes.[99] Through the introduction of hydrophilic succinic anhydride side groups onto the chitosan backbone, the water solubility of the natural polymer was significantly increased. Oxidation of alginate also led to an increased solubility of the polymer. Crosslinking between the two polymers was achieved through

a Schiff-base reaction followed by a lyophilization step. The Schiff-base reaction allowed for a reasonably slow sol-to-gel phase transition which in turn translated into a rehydrated gel scaffold that could easily be injected through a needle. During the rehydration step, the mRNA can be easily loaded into the system. When mRNA was used complexed to a nanoparticle system, a slower degradation rate of the gel was seen, when compared to empty gels, where a steadier release of the nucleic acids was measured overtime. These results were justified through additional crosslinking points occurring between the mRNA and the hydrogel polymer, therefore increasing the system overall stability. Regarding *in vivo* local mRNA-mediated protein expression, when using a Luc reporter gene, only the conditions where the injectable gel system was applied displayed local transfection *in vivo*. Transient expression of Luc was seen to peak at ~8 hr. Subsequently, OVA mRNA was used with the system to understand whether a relevant immune response could be triggered with the system. When looking into humoral immune responses using the injectable Chitosan-Alginate-OVA system, a significant increase in OVA-specific IgG levels was detected. An alternative injectable smart hydrogel (ISH) composed of HA functionalized with levodopa- and poly(ε-caprolactone-co-lactide)ester was developed for the delivery of OVA-expressing plasmid and GM-CSF for the local recruitment of DCs.[100] These displayed a slow degradation pattern while sustaining a controlled release of both polyplexes and GM-CSF *in vitro* and *in vivo*. The transfection efficiency assays showed that ISH were capable of effectively priming immune cells as seen through the expression of OVA in mice (Fig. 3a). Single subcutaneous injection of ISH in mice enhanced the recruitment of DCs, and of other immune cells, including macrophages and neutrophils. In turn, ISH generated strong antigen-specific humoral responses, and mice that received hydrogel-based vaccination did not develop tumors or had delayed tumor onset.

Efficient expansion of T-cells while avoiding T-cell exhaustion has been deemed a complex task that has captured the attention of the scientific community. Natural polymers have been applied in this regard. Alginate has been recently proposed for the development of a suspended culture method consisting of microscale hydrogel tubes (AlgTubes).[101] To evaluate the potential of the system, industry approved CD3/CD28 Dynabeads from Invitrogen and tetrameric anti-CD3/CD28/CD2 antibodies from Stem Cell Technologies were selected as controls. These tubes, while capable of protecting the cells from hydrodynamic stresses and compacting cells to ensure efficient cell mass transport, also created a cell-friendly microenvironment that allowed for high viability, low DNA damage, high growth rate, high purity, and high cellular yield when compared to the respective controls (Fig. 3b). Beyond the use of naturally sourced polymers for the *in vitro* expansion of T-cell, they have also been developed to deliver cells into resected tissue spaces or subcutaneously *via* small surgical procedure. A report has described the use of microporous alginate scaffolds together with a synthetic collagen-mimetic peptide (CMP) that binds to lymphocytes for biomaterial-supported lymphocyte delivery.[102] To promote the expansion of T-cells after implantation co-stimulatory cues, anti-CD3, anti-CD28, and anti-CD137 antibodies, were coupled to bilayered microspheres together with the superagonist IL-15 yielding a system that not only supports tumor-targeting T-cells but also reduces tumor relapse. Other similar systems have also emerged for the adoptive transfer of NK cells[103] and CAR T-cells.[104]

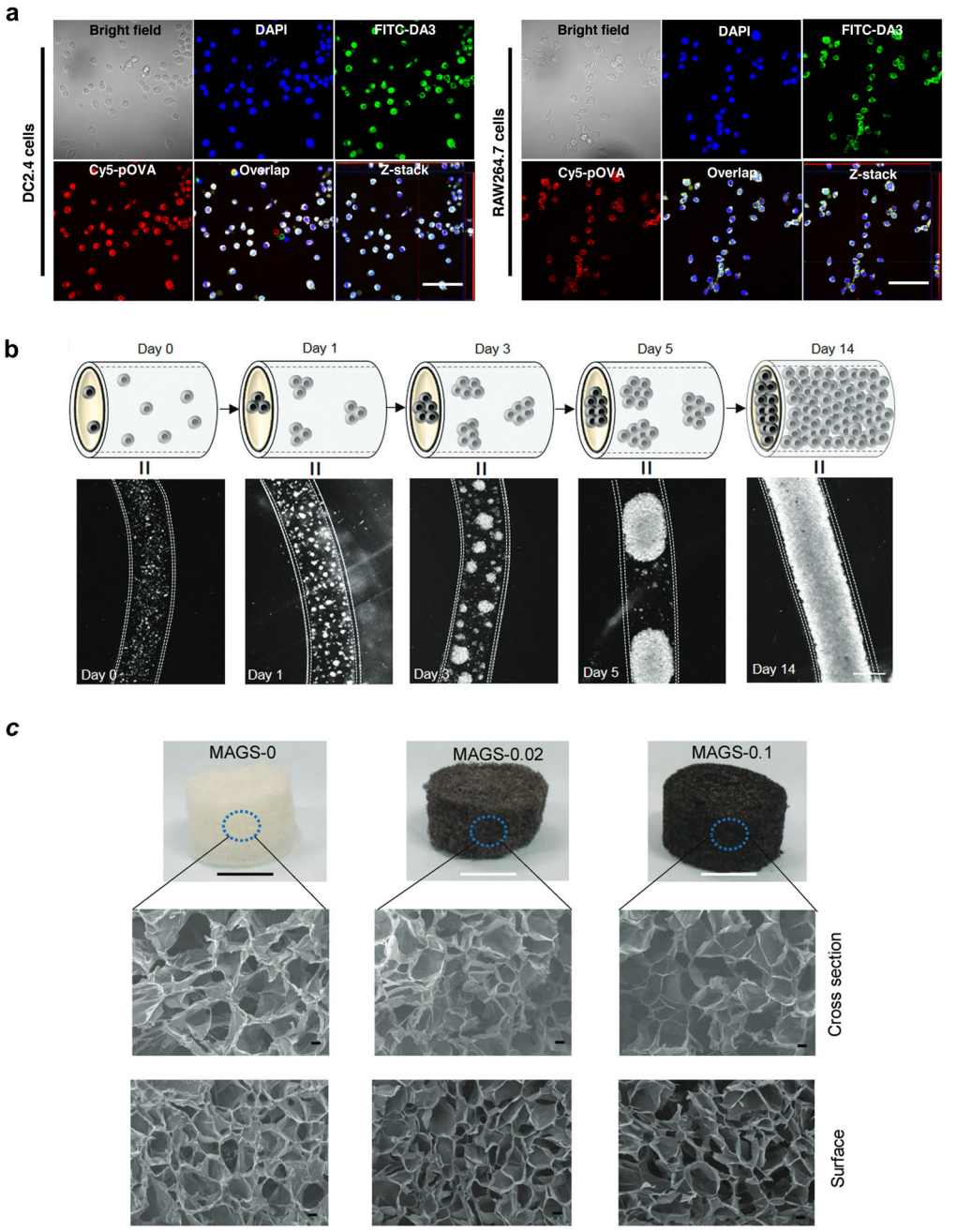

Figure 3. Natural-based hydrogels used in immunotherapeutic strategies. (a) Polyplexes released from the hydrogels induce priming of DC 2.4 mouse DCs and RAW 264.7. Adapted with permission from Ref.[100] Copyright 2020, Elsevier. (b) T cells cultured in AlgTubes first associate to form small clusters that subsequently grow until the tube is filled Adapted with permission from Ref.[101] Copyright 2018, John Wiley and Sons. (c) Characterization of alginate scaffolds containing various amounts of embedded MAGS. Adapted with permission from Ref.[105] Copyright 2019, John Wiley and Sons.

Alginate has also been used for the stimulation of autologous antigen-presenting cells, such as dendritic cells. Its capacity to be ionically cross-linkable has allowed for the production of 3D scaffolds embedded with reduced graphene oxide (MAGS) and loaded with OVA and GM-CSF that have been proposed as a vaccine delivery platform for *in situ* long-term activation of antigen-presenting DCs (Fig. 3c). MAGS were capable of efficiently loading both OVA and GM-CSF through direct pipetting onto the surface of the scaffold and the interaction between the reduced graphene oxide and the immunomodulators was strong enough to promote a slow and sustained release over time. When implanted subcutaneously, a significantly higher $CD11c^+$ DC recruitment was confirmed when GM-CSF-doped scaffolds were used, which was sustained over 30 days post-implantation. Antigen presentation efficiency was also increased when GM-CSF and OVA were co-loaded since the number of $CD11c^+$SIINFEKL-MHC-I^+ cells was significantly greater. This in turn was translated into a larger number of activated IFN-γ^+ $CD8^+$ T-cells and IFN-γ^+ $CD4^+$ T-cells in the lymph nodes of stimulated C57BL/6 mice. The MAGS-GM-CSF-OVA system also showed capability as a tool for vaccination. After completing 30 days post-vaccination, animals were challenged with OVA-expressing B16 melanoma (B16-OVA) cells and a delay in tumor growth was seen with high percentages of $CD11c^+CD86^+$ cells. When determining the effect of central and effector memory T-cells, mice that had been vaccinated with the system produced significantly higher numbers of these cells. Similarly to alginate, which is known for its biodegradability, the MAGS scaffolds showed *in vivo* a gradual loss of the interconnected structure over time, contributing to its biocompatibility in an *in vivo* setting.[105]

Growth-factor and cytokine encapsulation using tools, such as nanoparticle systems or drug-releasing hydrogels have in fact been widely used for tumor therapeutics development. However, over the past few years, increased focus has been placed on developing alternatives to these pre-loaded systems, such as cell encapsulation for the *in situ* production of cytokines at physiological concentrations. These living release systems could be used to either boost or improve immune responses or to directly combat tumors. Several of such immuno-protective cell encapsulation systems have already been reported. Alginate has been used in combination with K562 cells for the production and release of GM-CSF.[106] In other reports, for the release of IL6, genetically modified CHO cells were loaded into an alginate system to be used in a rat model of hepatocellular carcinoma.[107] Additionally, an alginate poly-L-lysinealginate (APA) copolymer was also reported for the microencapsulation of genetically modified mouse myoblasts (C2C12) for the delivery of angiostatin and IL-2 fusion protein (ssFvIL-2).[108] HEK293 cells were transfected with several cytokines and chemokine expression vectors for GM-CSF, IFNγ, and hIL-15 and encapsulated in alginate for further use in anti-tumor therapy experiments.[109] Atik et al. have described an HA-based low-viscosity hydrogel to serve as a vehicle for the delivery of tumor-specific CAR T-cells in convection-enhanced delivery (CED).[110] The hydrogel-based carrier presented a significantly higher CAR T-cell delivery rate when compared to saline while preserving the capacity of the CAR T-cells to migrate outward of the hydrogel and therefore exhibiting a significantly superior tumor-specific killing of glioma cells *vs.* saline after CED. Cellulose sulfate has been described as an encapsulation material for over 2 decades.[111] Cellulose when used as a mean of encapsulating mammalian cells, has been shown to protect cells from immune rejection, retain cells in the site of

implantation, provide long-term cell survival, and allow for the circulation of biomolecules.[112] A cellulose sulfate encapsulating system for Hut-78 cells to produce IL-2 for immunotherapeutic use has been reported.[113] Cellulose sulfate beads comprise a gelated membrane with interconnecting pores which restricts molecule circulation. Molecule properties, such as size, structure, flexibility, and charge end up playing a determinant role in their release from these particle systems. In this sense, small molecules have the upper hand regarding ease of escaping these 3D structures, while larger molecules are more dependent on their other characteristics. Salmons et al verified that upon stimulation with PMA and ionomycin, the encapsulated HUT-78 cells displayed the capacity to produce detectable levels of IL-2 as early as 3 days after encapsulation. Other authors have described similar systems comprised of gelatin hydrogels enzymatically crosslinked *via* microbial transglutaminase for the encapsulation of genetically engineered HEK293 to secrete human IL2.[114]

4.2. Microneedle-based systems

Vaccination is an established method to explore long-term immunization and is used to prevent a variety of pathologies of either bacterial or viral origin or even for the sensitization against TAAs in the case of cancer. Despite the well-known advantages of vaccination, needle-based immunization still presents issues, such as the risk of infection due to needle reuse and low patient compliance due to pain and fear. Microneedles (MNs) have been described as an optimal system for the dermal delivery of antigens as they can easily pierce the skin and deliver the antigen in the epidermis and dermis, ultimately in a pain-free delivery manner (Fig. 4). Mns take advantage of the several antigen-presenting cells present in skin, namely Langerhans cells (LCs) and dermal dendritic cells (dDCs).[115] These cells display the capacity to capture antigens and migrate to draining lymph nodes where they can present these antigens to T-cells, triggering a phenotypical switch to antigen-specific T-cells and B-cell activation. Dissolving microneedles (dMNs) in particular, consist of fast-dissolving excipients which can be polymers or sugars. When inserted in the skin, these needles dissolve and proceed to release bioactive compounds previously

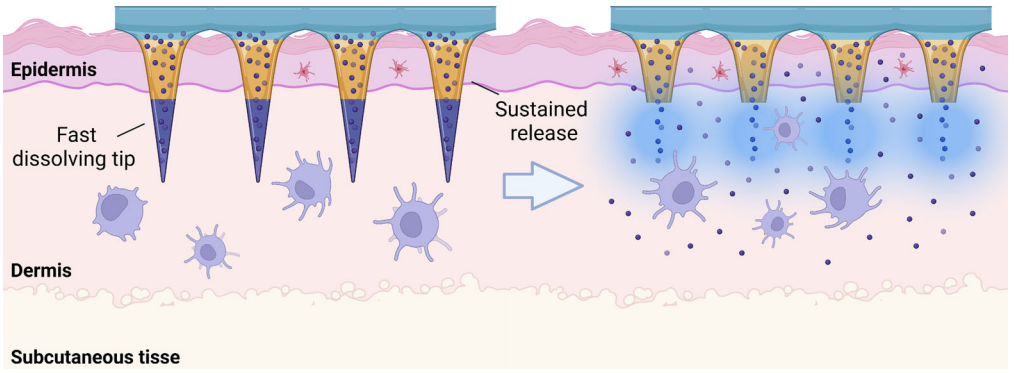

Figure 4. Representative scheme of a transdermal microneedle delivery system. A biphasic delivery system comprises a fast-dissolving biodegradable polymer tip, as the case of HA, which rapidly leads to the release of the desired molecule/proteins into the dermis or epidermis, followed by a slower biodegrading base that contributes to sustained release overtime.

added to the system making them a reliable system for intradermal (ID) vaccination. With this in consideration, several works have been recently proposed regarding microneedle-based approaches for vaccination. A chitosan MN system with a patch-dissolvable design has been reported for low-dose immunization.[116] Herein, an antigen-loaded chitosan MN was supported by an array patch of hydrophilic polyvinyl alcohol/polyvinylpyrrolidone (PVA/PVP) to provide additional strength upon MN insertion, overcoming the skin's inherent elasticity and deformation when exerting pressure. Upon complete insertion in the skin, the supporting array would then dissolve, reducing patch-induced skin irritation. This system showed that through ID implantation, a sustained release of -OVA was verified for up to 28 days. An *in vivo* rat model showed that a low-dose immunization with this system still led to persistently high antibody levels over an 18-week period which was deemed higher than that achieved through conventional intramuscular immunization. HA has been proposed for the development of dMNs for the ID delivery of OVA.[117] Through the fine-tuning of the loaded OVA peptide and the ratios of HA, a stable system was developed with an optimal penetration efficiency followed by gradual dissolution over a period of up to 20 min. When applied *in vivo* in the skin of female BALB/c (H2d) mice, an overall increase in IgG serum levels was detected. Another example of a recently developed microneedle vaccination system comprises a sodium hyaluronate (HA)/chitosan composite as the building block. This biphasic system was designed to allow for both a rapid and sustained release of antigens mimicking more conventional prime-boost immunization regimen when compared to traditional bolus injection or the previously described chitosan MN alone. For this effect, the authors developed an MN system composed of an antigen-loaded HA tip and chitosan base, which was combined with a PVA/PVP supporting structure.[118] The HA/chitosan composite MN when inserted in porcine cadaver skin and rat skin showed to pierce through the stratum corneum and reach the dermal layer. The dissolvable HA tip dissolved within the skin for rapid release of the encapsulated antigens, therefore priming the immune system, while the biodegradable chitosan base remained in the dermis for a prolonged antigen-release over 4 wk, further boosting the antigen-driven responses for up to 16 wk. Immunization with the HA/chitosan MN containing OVA was shown to stimulate both Th1 and Th2 immune responses in Sprague – Dawley rats when compared to more traditional two-dose or double-dose subcutaneous vaccination. Another MN immunotherapy system has been developed for the transdermal delivery of tumor antigens aided by near-infrared (NIR) light emission. Herein, a methacrylated hyaluronic acid solution was used together with GM-CSF, homogenized B16F10 tumor lysate, and melanin to make the MN system.[119] Melanin when combined with NIR led to heat generation which in turn promoted tumor-antigen uptake by DCs that were locally driven by the release of GM-CSF.

Additional works making use of the biodegradable and hydrogel-forming properties of HA and chitosan for the development of microneedle delivery systems have also been reported.[120–124]

4.3. Film-based systems

As aforementioned, the topical delivery of proteins is a good alternative to more traditional invasive delivery methods and while natural-based polymers, such as GG have

been explored to develop films for the dressing of early-stage cancer or as wound care after surgery in late-stage of oral cancer treatment,[125] ID through intact skin comes not without its own setbacks, as it may be rather restrictive depending on the size, stability or even hydrophilic properties of the molecules to be delivered. A method to overcome some of these issues resides in the use of fractional ablative laser microporation, in which pulsed infrared lasers are used to induce a thermal ablation of tissue in micron-sized columns with a diameter of 30–200 µm. Engelke et al.[126] described water-soluble dry films to be used as a drug delivery system in laser microporated skin (Fig. 5). Blends of PVA with carboxymethyl cellulose (CMC) were used for the production of a hydrophilic polymer with the purpose of solubilization of the films directly on laser microporated skin, given the enhanced water transport from the tissue through the porated skin into the film under occlusion. The PVA/CMC blend films were shown to dissolve within <6 hr when attached on top of laser-generated micropores, facilitated by the generation of a liquid depot between the skin surface and the occlusive tape used for film fixation, which in turn led to the efficient delivery of both RD70 and PS-particles into and through excised pig skin.[126] While the application of such natural polymers for the production of immunotherapeutic films hasn't been explored extensively, the use of these polymers for the transdermal delivery of drugs has resulted in several bodies of work that can easily be adapted for immunotherapeutics. Some of these rely on combinations of chitosan with GG,[127] HA,[128] or PVA.[129]

4.4. Nanotubes-based systems

The use of chitosan as an adjuvant for photothermal therapy (PTT) was recently described.[130] For the effect, previously a single-walled carbon nanotube (SWNT) modified with glycated chitosan (GC) system had been reported[131] (Fig. 6a). This system was then used was used in combination with α-CTLA4 as an anti-tumor therapy strategy (Fig. 6b). This combinatorial treatment was capable of significantly increasing ROS production in 4T1 tumor cells, despite not displaying any direct cytotoxic activity. However, results showed that by the combined use of the SWNT-GC with laser irradiation a significant increase in killing of 4T1 tumor cells was visible, which resulted from

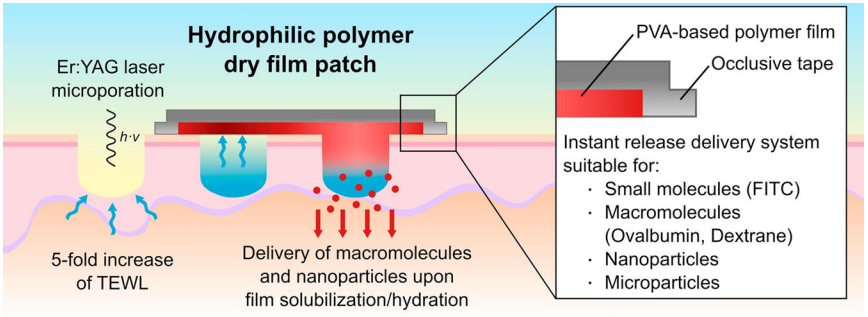

Figure 5. Schematic representation of the use of polymer-based films as a transdermal immunotherapeutic strategy. Water-soluble dry films were developed through blends of PVA, namely with chemically modified cellulose. These films are solubilized when applied directly on microporated skin, facilitated by enhanced water transport from the tissue, which allows for the delivery of small molecules and antigens of interest. Adapted with permission from Ref.[126] Copyright 2018, Elsevier.

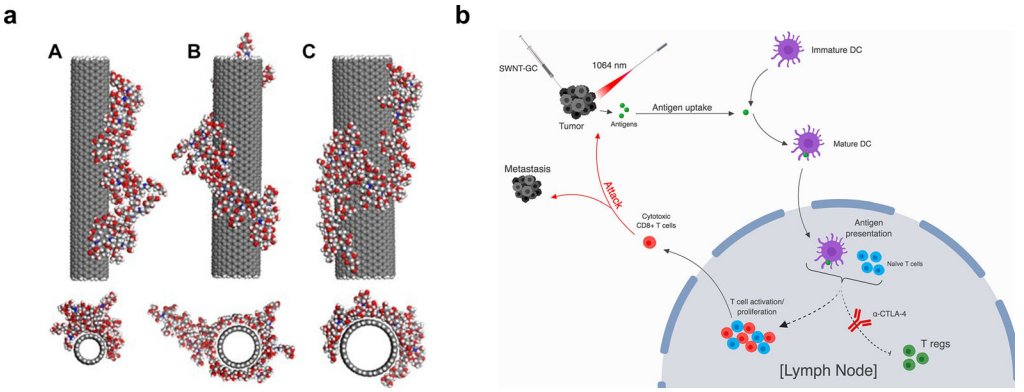

Figure 6. Application of SWNT as a delivery method of GC's aided by laser irradiation. (a) Simulation snapshots of the GC adjuvants wrapped around SWNTs with different diameters, (A) 12.20, (B) 16.27, and (C) 20.34 Å, respectively. Adapted under the terms of license CC BY-NC 4.0 from Ref.[131] Copyright 2018, the authors. (b) Schematics representing a laser immunotherapy system for the effective treatment of metastatic cancers. SWNTs functionalized with GC are co-administered with checkpoint inhibitors, anti-CTLA-4 antibodies, in conjunction with laser irradiation to enhance tumor antigen uptake and presentation. Adapted with permission from Ref.[130] Copyright 2019, Elsevier.

a more pronounced temperature increase when SWNT-GC was used in comparison to laser alone. Regarding the immunomodulatory effect of the system, when SWNT-GC was placed in a culture with dendritic cells, a higher increase in the expression of CD40 and CD80 could be verified by flow cytometry. Moreover, in the presence of 4T1 tumor cells, an even higher DC activation could be verified as well as increased secretion of TNF-α. When tested in tumor-bearing mice, not only did the SWNT-GC system lead to a higher temperature increase inside the tumor under laser irradiation but also lead to a significant reduction in tumor size and number of metastases. Ultimately, when combined with checkpoint inhibition, a significant increase in mice survival time was verified as well as in the production of IFN-γ by splenocytes.

4.5. Particle-based systems

One of the main features of chitosan as a base system for the development of immunotherapeutic strategies is its spontaneous self-assembly in micro- or nanoparticles when ionically crosslinked with charges of opposite nature. Complexes of different natures may also be formed when using anionic crosslinking substrates. Recently, the effect of chitosan nanoparticles on Vγ9Vδ2 T-cells has been studied.[132] This particular subset of T-cells was shown to recognize stress-induced phosphonate antigens presented by both cancer cells and pathogen-infected cells in an MHC-independent manner. Chitosan nanoparticles where shown, as a standalone system, to be capable of enhancing the killing potential of Vγ9Vδ2 T-cells *via* upregulation of killing molecule NKG2D expression as well as of FasL and CD56. This system was also shown to have immunomodulatory properties through the enhancement of perforin secretion which is involved in cytotoxic T-cell responses. Ultimately, killing potential was confirmed *in vitro* through direct culture with leukemia cells, which showed an increased killing rate of Vγ9Vδ2 T-cells when pretreated with the chitosan nanoparticle system. Other studies used this natural

polymer for the development of antigen delivery tools in the form of chitosan-modified poly(lactic-co-glycolic acid) (PLGA) nanoparticles (CS-AHPP/OVA).[133] While retaining excellent stability, these particles were shown to induce strong cellular immune responses as seen through the evaluation of lymphocyte proliferation, while also being capable of inducing the secretion of IFN-γ and TNF-α. T-cell polarization was also assessed through the production of IgG1 and IgG2a antibodies associated, respectively Th2 and Th1-polarized immune responses. Both IgG1 and IgG2a antibodies were highly induced upon stimulation with the CS-AHPP/OVA system. The potential use of chitosan as an effective adjuvant for DNA vaccines was also explored through the development of a chitosan nanoparticle comprising human papilloma virus (HPV)-16 E7 DNA and IL-12 gene.[134] Chitosan nanoparticles as a standalone system have been shown to display immunostimulatory activity.[135] Moreover, their use as co-adjuvants for cytokine therapy, was proven to increase local cytokine retention and bioactivity.[136] The chitosan-E7 DNA + IL-12 system was shown to enhance the proliferative response of T-cells to the system. Moreover, an increased cytolytic activity was verified by lactate dehydrogenase (LDH) assay. Mice immunized with this system also presented a significantly higher production of IFN-γ and IL-4 and a decrease in the production of the immunosuppressive cytokine IL-10. When looking into the *in vivo* effect in a mouse tumor model, a reduction in tumor volume was seen upon immunization with chitosan-E7 DNA + IL-12.[134] Additional chitosan-based adjuvant systems have been presented. Choi et al., described a Chitosan-RNA adjuvant system for immune modulation where nanoscale polyplexes of TLR-3-recognizing RNA adjuvants and high molecular weight chitosan (RA/CTS) were formed by ionic crosslinking.[137] Through the subcutaneous injection of the RA/CTS polyplexes in an OVA tumor mouse model, it was possible to show that this system exerted a preventative effect upon challenge with B16-OVA cell line. A greater inhibitory effect was also verified when a second challenge was performed in comparison with other treatments, which was associated with a higher tumor antigen-specific humoral and cellular immune response and with a greater infiltration of CD4 helper T-cells as well as CD8 T-cells into tumor tissues. Other reports have also used chitosan as a delivery system for RNA-targeting cancer therapy. Chitosan-coated selenium nanoparticles (SeNPs) with a folic acid targeting moiety were developed for Fluc mRNA delivery to cancer cells.[138] These particles were capable of not only successfully binding mRNA but also of conferring significant protection to the nucleic acids. This allowed for a stable delivery of the mRNA cargo when tested *in vitro*, and a further uptake of the FA-targeting system when tested in folate receptor-positive cells. Additionally, other studies have shown that chitosan may reduce the pro-oxidative activity of selenium, which was shown to lead to DNA damage. A polyethylene glycol (PEG)-chitosan-lactate (PCL) nanoparticle system has recently been developed for the delivery of A2AR-specific mRNA,[139] which is known to interfere with the differentiation and function of T-cells and has also been associated with significant tumor regression in tumor-bearing mice.[140] This A2AR siRNA delivery system displayed a high transfection efficiency in T-cells while yielding low toxicity in the several cell lines that were tested and an elevated concentration in the tumor zone when biodistribution was tested. T-cells stimulated with the A2AR siRNA-loaded nanoparticles demonstrated a suppressed expression of A2AR which in turn was associated with increased T-cell

proliferation, reduced apoptosis, and increased production of IFN-γ while reducing the secretion of inhibitory cytokine IL-10. Additionally, differentiation of T-cells into Treg's was blocked by using A2AR siRNA-loaded nanoparticles in tumor-bearing mice.

Block co-polymerization has been extensively applied in the field of TERM to develop more advanced materials with more appealing characteristics and features. Block co-polymers possess a linear arrangement of "blocks" of repeating units with varying monomer compositions. Recently, this technology has made its way into immunotherapy for the development of new delivery systems. A novel co-polymer consisting of sodium alginate modified with β-cyclodextrin (Alg-β-CD), methoxypolyethylene glycol (mPEG-Fc) containing ferrocene (Fc), and α-cyclodextrin (α-CD) was developed. The non-covalent co-polymer Alg-β-CD/mPEG-Fc/α-CD was then self-assembled into nanoparticles for controlled drug delivery, as these nanoparticles display the capacity to disassemble in the presence of H_2O_2.[141] This is relevant since glucose oxidase (GOD) can oxidize glucose to produce H_2O_2 and has been tied to tumorigenesis.[142] To determine the capacity of the system to be used for controlled delivery, bovine serum albumin (BSA) was added to the mixture before the complexation with α-CD yielding BSA-loaded nanoparticles. A high degree of encapsulation was confirmed in the Alg-β-CD/mPEG-Fc/α-CD when compared to the β-CD free counterpart. Regarding release profile, when tested in a solution fitted with GOD and glucose, the Alg-β-CD/mPEG-Fc/α-CD/BSA shared a significantly higher release of pilot molecule BSA.

The combinatorial use of inorganic materials with natural origin polymers has been used to achieve certain relevant properties otherwise unachievable. Given the interaction of HA with its receptor CD44, metal-organic frameworks have been functionalized with HA for the delivery of hypoxia inducible factor signaling inhibitor (ACF) and CpG[143] (Fig. 7a). As expected, a targeted delivery to cancer cells overexpressing the CD44 receptor was achieved as well as increased internalization. This system was applied in the context of photodynamic therapy which is known to aggravate tumor hypoxia therefore leading to tumor survival and metastasis of remaining cancer cells through the upregulation of certain factors, such as VEGF, B-cell lymphoma-2 (BCL-2), and metalloproteinase (MMP)-9. When applying the PCN-ACF-CpG@HA system, a decrease of these factors was confirmed which in turn led to a significantly lower cell viability upon laser irradiation. Furthermore, tumor suppression increased levels of intra-tumoral IL-12p70, IFN-γ, and TNF-α, and ultimately more infiltrating $CD8^+$ and $CD4^+$ T-cells were confirmed at the tumor site. Several other systems have been developed with the targeting capabilities of HA in mind. Reports have explored the use of HA for the coating of novel polypyrrole nanoparticles using the near-infrared dye IRDye800CW with camptothecin (CPT) for synergistic chemo-PTT[144] (Fig. 7b). These particles showed enhanced tumor targeting when used in combination with laser irradiation and anti-PD-L1 immunotherapy, complete tumor eradication, with no recurrence during the entire 24-day observation period, could be seen, while no lung metastasis were found. Additionally, levels of immunomodulatory cytokines TNF-α, IFN-γ, IL-1β, IL-2, IL-4, and IL-6 were significantly increased when treated by this method. Other strategies describe the use of HA for the coating of a liposomal nanoparticle system for the targeting of tumor-expressing CD44 cells based on synthetic aminoxy lipids,[145] which showed an increased internalization over 24 hr when tested in CD44 expressing tumor cell lines (human non-small cell lung carcinoma H1299

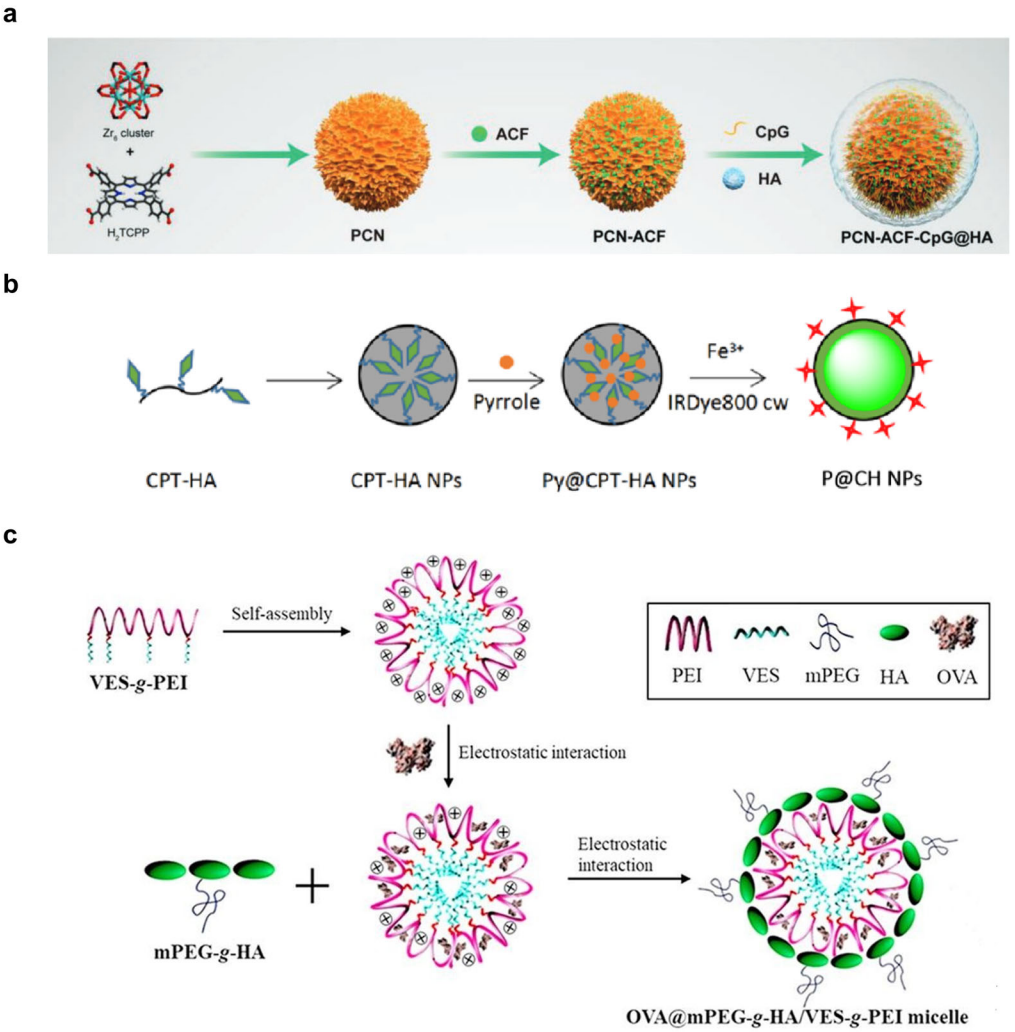

Figure 7. HA as an adjuvant for the development of nanoparticles for immunotherapy. (a) Concept for the preparation of PCN-ACF-CpG@HA nanoparticles to be used as an *in situ* tumor vaccine by integrating PDT, antihypoxic signaling, and CpG adjuvants. Adapted with permission from Ref.[143] Copyright 2019, John Wiley and Sons. (b) Illustration of the formation of CD44 protein targeting P@CH nanoparticles, capable of combined chemo-photothermal therapy. Adapted with permission from Ref.[144] Copyright 2019, Elsevier. (c) Schematic illustration of the production of mPEG-g-HA/VES-g-PEI micelle for the targeted delivery of OVA and the triggering of CTL-mediated killing of tumor cells. Adapted with permission from Ref.[147] Copyright 2019, Elsevier.

and cervical carcinoma HeLa cells). Extracellular vesicles (EV) are known as an optimal system for the delivery of biomolecules as they originate from the cell membrane and therefore display fewer side effects than synthetic counterparts like liposomes. An EV-based vaccine system, referred to as HDEA@EVAT, has been achieved through coupling with HA, 3-(diethylamino)propylamine (DEAP), the immunomodulator monophosphoryl lipid A (MPLA) and mucin 1 (MUC1) antigen.[146] This system displayed a DC targeting potential derived from the high expression of CD44 in these cells, which led to improved endocytosis rates and hence a greater DC activation rate seen by increased

CD86 levels and in turn translated into increased levels of TNF-α followed by higher levels of IFN-γ production by $CD8^+$ T-cells. Other vaccine systems have also been developed considering the affinity of HA toward CD44. This was the case of pegylated HA which has been developed for the coating of multifunctional micelles loaded with OVA peptide[147] (Fig. 7c). These antigen delivery micelles when functionalized with HA-induced high cellular uptake by B16-F10 cells when compared to non-modified particles, thus proving once again CD44 targetability. As CD44 may be expressed on the surface of several cells, other works have taken advantage of this for the targeting of macrophages. Recently, HA-decorated superparamagnetic iron oxide nanoparticles (HIONs) have been developed for the artificial reprogramming of macrophages,[148] thus allowing for a higher particle internalization into macrophages leading to increased production of inflammatory factors NOS and TNF-α and increased levels of $CD80^+$ macrophages. Additionally, when tested *in vivo*, HION-reprogrammed macrophages were capable of reeducating neighbor M2 phenotype to shift toward activated M1 macrophages mainly due to the cell-to-cell communication.

The targeted delivery of Granzyme B (GrB) has trigged interest due to its cytotoxic efficacy and action in a variety of apoptosis-inducing mechanisms.[149] Considering the alluring properties of HA for the delivery of key molecules in the tumor microenvironment, nanoparticles have been developed for the targeted delivery of GrB containing a cell-penetrating peptide TAT (GrB-T) capable of inducing cell apoptosis.[150] *In vivo*, this system showed that this mechanism led to the extracellular release of GrB-T, which enters the cell cytoplasm and triggers subsequent extrinsic apoptosis pathways, resulting in a significant anti-tumor effect. Other systems have embraced the same concept to take advantage of the biodegradable nature of HA. A nanoplatform-based system comprised of Chlorin e6 (Ce6)-conjugated HA, dextro-1-methyl tryptophan (1-mt)-conjugated polylysine (PM), and anti-PD-L1 monoclonal antibodies was developed[151] as a technique to tackle, in one step, the 3 pathways that comprise the immunological cascade (antigen presentation, lymphocyte activation and proliferation/differentiation, and tumor elimination. In this case, the enzyme rich tumor microenvironment triggered the release of the indoleamine 2,3-dioxygenase (IDO) inhibitor 1-mt and anti-PD-L1. This, in turn, promoted DC maturation, lymphocyte activation, the inhibition of the IDO pathway (enhancement of proliferation/differentiation), and the blocking of the PD-1/PD-L1 pathway (boosted tumor elimination), ultimately leading to an increased survival rate of tumor-induced mice.

Imidazoquinolines (IMQs) have become of interest in the immunological field due to their ability to activate TLR-7 and TLR-8, which in turn induces the secretion of pro-inflammatory cytokines that promote innate immune responses. However, while the systemic administration of these compounds has not yet been approved, a need for novel systems capable of delivering IMQs while reducing systemic inflammation and toxicity has been identified. For this purpose, HA has been conjugated to tocopherol (vitamin E) to be used as a nanocarrier for the delivery of the R848-Toco prodrug.[152] When tested *in vitro*, the R848-Toco/HA-Toco system triggered a higher TLR-7 activity when compared to controls with higher levels of secreted TNF-α. Upon *in vivo* administration in a tumor mouse model, R848-Toco/HA-Toco significantly suppressed tumor growth when compared to an HA-Toco vehicle over time and generated an increase of CD8a, CD11c, and CD11b.

Combination therapy, which consists of the simultaneous administration of traditional chemotherapeutics with novel immunotherapy strategies, is being explored for application in cancer therapy. Systems comprising natural polymers with biodegradable properties are bring used in the development of novel and more advanced forms of applying these combinatorial therapeutics. A nanoparticle-based doxorubicin (DOX) delivery system with an MMP-sensitive peptide (CPLGLAGG) for enzyme-activated drug release comprising a HA tumor targeting moiety (HA-Psi-DOX) was described[153] to be used in combination with anti-PD-1L therapy. When tested *in vivo*, the system showed a clear homing ability, while also presenting a good retention ability when in circulation. This, in turn, translated into increased intra-tumoral content of IFN-γ and PD-L1, which ultimately led to significant inhibition of tumor growth, a higher number of tumor-infiltrating lymphocytes, and an improved antimetastatic capability.

Natural polymers can many times be heavily modified to yield synthetic complexes with biological response-modifying properties. Such is the case of cellulose, which has been used to develop the TLR-3 agonist Poly-ICLC (Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose).[154] Poly ICLC is a synthetic double-stranded RNA complex used as an immunostimulant and has been tested over several studies and clinical trials (Clinicaltrials.gov Identifier's: NCT01984892, NCT03721679) and more recently has been used in combination with Flt3L and radiotherapy to yield an *in situ* vaccine (ISV) currently undergoing clinical testing (Clinicaltrials.gov Identifier: NCT01976585).[155] Pre-clinical settings showed that upon treatment with the ISV, a marked accumulation of intratumoral cross-presenting DC's was confirmed, which in turn led to a higher uptake of TAAs generating systemic tumor-specific $CD8^+$ T-cell responses. Through the combinatorial effect of PD-1 blockade, an increased remission rate of up to 40% was verified. Patients with Indolent Non-Hodgkin's Lymphoma enrolled in the phase I trial displayed similar expansion of DC subsets, upregulation of checkpoint molecules, and durable regressions of distant (untreated) tumors.

5. The future of natural polymers in cancer immunotherapy

While combination therapy has created advances in the field of immunotherapy through the requirement of more advanced forms of antigen presentation, key issues, such as the susceptibility for proteolytic degradation of immunotherapeutics as the case of CAR ubiquitination[156,157] or even the effect of microenvironment immunosuppression,[158–160] has raised concerns in the field. And while efforts have been made to attenuate several concerns in the use of immunotherapeutics as minimizing off-target effects and reducing off-tumor toxicity through the development of novel systems as the case of probodies,[161–163] CAR masking[164] or even through the development of hypoxia-responsive CAR T-cells,[165] issues continue to appear when attempting different therapeutic strategies over the course of the disease.

This has opened the door for new developments based on technologies typically used in other areas like polymer and tissue engineering. A chief example is the creation of polymer-based advanced delivery systems to aid in shielding biomolecules from the microenvironment.[166–168]

The introduction of natural polymers came naturally due to several advantages associated with them, namely their biodegradability, biocompatibility, accessibility, little foreign body response, and interaction with adhesive receptors and cell signaling[169] ultimately overcoming synthetic polymers. However, despite the already significant presence of natural polymers in the field of immunotherapy, their use will grow even more in the future. As seen throughout this overview, several novel systems based on natural polymers have been recently developed and while several of these systems still comprise blends with synthetic polymers or are used in a combinatorial fashion, this marks a starting point of transition toward a broader use of naturally occurring polymers.

Many of these systems are still at a proof-of-concept stage using test molecules like -OVA to assess basic biological behavior.[170–173] Upon *in vitro* and *in vivo* assessment of their efficacy with TAA, some of these systems may be in clinical trials in the next few years in areas like vaccination. Certain natural polymers like chitosan and alginate salts can in fact serve as great natural platforms to develop vaccination tools due to their action as natural immunoadjuvants.[91,170,174,175] Furthermore, hybrid systems taking advantage of key features of different polymers, natural or otherwise, will constitute an important portion of what the field of polymer engineering may give to immunotherapy.

As a better understanding of the basic mechanisms underlying immune-associated pathologies is achieved, more efficiently well-known or even unexplored natural polymers may be applied and new hybrid polymer systems created. Such example is the, repeated amount of interest that has spiked around HA and its affinity for CD44 and RHAMM allowing the development of tumor-targeting drug delivery systems and immunotherapeutics[176–179] and hence compelling the necessity to uncover unknown interactions of natural polymers with biological systems.

While many of the aforementioned systems throughout this review are still in what is considered an early stage of development, several polymer-based systems have already made into clinical trials as the case of BP-C2 which makes use of the natural polymer liganin (Clinicaltrials.gov Identifier: NCT04186585), IP-001 which consists of 1% N-dihydro-galacto-chitosan (Clinicaltrials.gov Identifier: NCT03993678) or the administration of GC (Clinicaltrials.gov Identifier: NCT03202446). Additionally, having some of these natural polymers already been approved by the FDA for different applications as the example of chitosan in Axiostat® for use as a hemostatic, modified cellulose as in the case of AQUACEL® dressing or even HA which has been widely used in the cosmetic industry for several years and allows for a faster clinical translation of some of these systems as several safety requirements have been already proved.

While it is crucial to produce qualitative data that supports the advance of these systems to clinical trials, it is also important to keep in mind manufacturing issues that may take place when attempting to upscale many of these systems when developing these novel therapeutics. Batch-to-batch variability is a concern that must be addressed to streamline these naturally-sourced polymers into clinical application. Additional issues like failure in simulating original laboratory conditions, complex experimental design that may hamper large-scale manufacturing processes, and difficulty in meeting quality control standards for clinical use are just a few. Therefore, it is critical that these issues are considered when designing platforms to be translated clinically to the bedside.

Therefore, adopting the use of natural polymers to develop new systems or further enhance currently used ones brings clear benefits. The exploration of novel sources of these polymers, such as marine-based natural polymers,[180,181] will open the doors to the creation of new materials with novel properties and characteristics that will further advance the field. However, one must keep in mind that this novelty comes with a tradeoff regarding the required path for regulatory approval. Therefore, while an effort to source even more accessible and cheaper polymers from different natural origins is welcomed, it must be accompanied by a matching effort to establish their safety in pre-clinical and clinical trials.

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References

1. Xiao, Y.; Yu, D. Tumor Microenvironment as a Therapeutic Target in Cancer. *Pharmacol. Ther.* **2021**, *221*, 107753. DOI: 10.1016/j.pharmthera.2020.107753.
2. Tiwari, A.; Trivedi, R.; Lin, S.-Y. Tumor Microenvironment: Barrier or Opportunity towards Effective Cancer Therapy. *J. Biomed. Sci.* **2022**, *29*, 83. DOI: 10.1186/s12929-022-00866-3.
3. Dzobo, K.; Senthebane, D. A.; Dandara, C. The Tumor Microenvironment in Tumorigenesis and Therapy Resistance Revisited. *Cancers* **2023**, *15*, 376. DOI: 10.3390/cancers15020376.
4. Wherry, E. J. T Cell Exhaustion. *Nat. Immunol.* **2011**, *12*, 492–499. DOI: 10.1038/ni.2035.
5. DeVito, N. C.; Plebanek, M. P.; Theivanthiran, B.; Hanks, B. A. Role of Tumor-Mediated Dendritic Cell Tolerization in Immune Evasion. *Front. Immunol.* **2019**, *10*, 2876. DOI: 10.3389/fimmu.2019.02876.
6. Leach, D. R.; Krummel, M. F.; Allison, J. P. Enhancement of Antitumor Immunity by CTLA-4 Blockade. *Science* **1996**, *271*, 1734–1736. DOI: 10.1126/science.271.5256.1734.
7. Reddy, M. S.; Ponnamma, D.; Choudhary, R.; Sadasivuni, K. K. A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds. *Polymers* **2021**, *13*, 1105. DOI: 10.3390/polym13071105.
8. Deng, G.; Wong, W.-T.; Huang, M.; Wu, R.; Lai, W.-F. Chapter 8 – Self-Healing Properties of Hydrogels Based on Natural Polymers. In *Hydrogels Based on Natural Polymers*; Chen, Y. B. T.-H. B. on N. P., Ed.; Elsevier: Netherlands, **2020**; pp 223–245. DOI: 10.1016/B978-0-12-816421-1.00008-2.
9. Razavi, S. M. R.; Oh, J.; Sett, S.; Feng, L.; Yan, X.; Hoque, M. J.; Liu, A.; Haasch, R. T.; Masoomi, M.; Bagheri, R.; et al. Superhydrophobic Surfaces Made from Naturally Derived

Hydrophobic Materials. *ACS Sustain. Chem. Eng.* **2017**, *5*, 11362–11370. DOI: 10.1021/acssuschemeng.7b02424.

10. Ghalia, M. A.; Abdelrasoul, A.; 7 – Compressive and Fracture Toughness of Natural and Synthetic Fiber-Reinforced Polymer. In *Fibre-Reinforced Composites and Hybrid Composites, Woodhead Publishing Series in Composites Science and Engineering*; Jawaid, M., Thariq, M., Saba, N. B. T.-M. and P. T. of B., Eds.; Woodhead Publishing: United Kingdom, **2019**; pp 123–140. DOI: 10.1016/B978-0-08-102292-4.00007-2.
11. Esfahani, K.; Roudaia, L.; Buhlaiga, N.; Del Rincon, S.; V; Papneja, N.; Miller, W. H. A. Review of Cancer Immunotherapy: From the Past, to the Present, to the Future. *Curr. Oncol.* **2020**, *27*, 87–97. DOI: 10.3747/co.27.5223.
12. Kimiz-Gebologlu, I.; Gulce-Iz, S.; Biray-Avci, C. Monoclonal Antibodies in Cancer Immunotherapy. *Mol. Biol. Rep.* **2018**, *45*, 2935–2940. DOI: 10.1007/s11033-018-4427-x.
13. Berraondo, P.; Sanmamed, M. F.; Ochoa, M. C.; Etxeberria, I.; Aznar, M. A.; Pérez-Gracia, J. L.; Rodríguez-Ruiz, M. E.; Ponz-Sarvise, M.; Castañón, E.; Melero, I. Cytokines in Clinical Cancer Immunotherapy. *Br. J. Cancer* **2019**, *120*, 6–15. DOI: 10.1038/s41416-018-0328-y.
14. Guermonprez, P.; Valladeau, J.; Zitvogel, L.; Théry, C.; Amigorena, S. Antigen Presentation and T Cell Stimulation by Dendritic Cells. *Annu. Rev. Immunol.* **2002**, *20*, 621–667. DOI: 10.1146/annurev.immunol.20.100301.064828.
15. Restifo, N. P.; Dudley, M. E.; Rosenberg, S. A. Adoptive Immunotherapy for Cancer: Harnessing the T Cell Response. *Nat. Rev. Immunol.* **2012**, *12*, 269–281. DOI: 10.1038/nri3191.
16. Brenner, M. K.; Heslop, H. E. Adoptive T Cell Therapy of Cancer. *Curr. Opin. Immunol.* **2010**, *22*, 251–257. DOI: 10.1016/J.COI.2010.01.020.
17. Geethakumari, P. R.; Ramasamy, D. P.; Dholaria, B.; Berdeja, J.; Kansagra, A. Balancing Quality, Cost, and Access During Delivery of Newer Cellular and Immunotherapy Treatments. *Curr. Hematol. Malig. Rep.* **2021**, *16*, 345–356. DOI: 10.1007/s11899-021-00635-3.
18. Gomes-Silva, D.; Ramos, C. A. Cancer Immunotherapy Using CAR-T Cells: From the Research Bench to the Assembly Line. *Biotechnol. J.* **2018**, *13*, 1700097. DOI: 10.1002/biot.201700097.
19. Sprent, J.; Surh, C. D. Normal T Cell Homeostasis: The Conversion of Naive Cells into Memory-Phenotype Cells. *Nat. Immunol.* **2011**, *12*, 478–484. DOI: 10.1038/ni.2018.
20. Zhang, N.; Bevan, M. J. $CD8^+$ T Cells: Foot Soldiers of the Immune System. *Immunity* **2011**, *35*, 161–168. DOI: 10.1016/j.immuni.2011.07.010.
21. Takada, K.; Jameson, S. C. Naive T Cell Homeostasis: From Awareness of Space to a Sense of Place. *Nat. Rev. Immunol.* **2009**, *9*, 823–832. DOI: 10.1038/nri2657.
22. Ma, A.; Koka, R.; Burkett, P. Diverse Functions OF Il-2, Il-15, and Il-7 IN Lymphoid Homeostasis. *Annu. Rev. Immunol.* **2006**, *24*, 657–679. DOI: 10.1146/annurev.immunol.24.021605.090727.
23. Malek, T. R. The Biology of Interleukin-2. *Annu. Rev. Immunol.* **2008**, *26*, 453–479. DOI: 10.1146/annurev.immunol.26.021607.090357.
24. Spolski, R.; Leonard, W. J. Interleukin-21: Basic Biology and Implications for Cancer and Autoimmunity. *Annu. Rev. Immunol.* **2008**, *26*, 57–79. DOI: 10.1146/annurev.immunol.26.021607.090316.
25. Mescher, M. F.; Curtsinger, J. M.; Agarwal, P.; Casey, K. A.; Gerner, M.; Hammerbeck, C. D.; Popescu, F.; Xiao, Z. Signals Required for Programming Effector and Memory Development by $CD8^+$ T Cells. *Immunol. Rev.* **2006**, *211*, 81–92. DOI: 10.1111/j.0105-2896.2006.00382.x.
26. Arakaki, R.; Yamada, A.; Kudo, Y.; Hayashi, Y.; Ishimaru, N. Mechanism of Activation-Induced Cell Death of T Cells and Regulation of FasL Expression. *Crit. Rev. Immunol.* **2014**, *34*, 301–314. DOI: 10.1615/critrevimmunol.2014009988.
27. Green, D. R.; Droin, N.; Pinkoski, M. Activation-Induced Cell Death in T Cells. *Immunol. Rev.* **2003**, *193*, 70–81. DOI: 10.1034/j.1600-065X.2003.00051.x.

28. Klein, E.; Ben-Bassat, H.; Neumann, H.; Ralph, P.; Zeuthen, J.; Polliack, A.; Vánky, F. Properties of the K562 Cell Line, Derived from a Patient with Chronic Myeloid Leukemia. *Int. J. Cancer* **1976**, *18*, 421–431. DOI: 10.1002/ijc.2910180405.
29. Butler, M. O.; Lee, J.-S.; Ansen, S.; Neuberg, D.; Hodi, F. S.; Murray, A. P.; Drury, L.; Berezovskaya, A.; Mulligan, R. C.; Nadler, L. M.; et al. Long-Lived Antitumor $CD8^+$ Lymphocytes for Adoptive Therapy Generated Using an Artificial Antigen-Presenting Cell. *Clin. Cancer Res.* **2007**, *13*, 1857–1867. DOI: 10.1158/1078-0432.CCR-06-1905.
30. Paczesny, S.; Banchereau, J.; Wittkowski, K. M.; Saracino, G.; Fay, J.; Palucka, A. K. Expansion of Melanoma-Specific Cytolytic $CD8^+$ T Cell Precursors in Patients with Metastatic Melanoma Vaccinated with $CD34^+$ Progenitor-Derived Dendritic Cells. *J. Exp. Med.* **2004**, *199*, 1503–1511. DOI: 10.1084/jem.20032118.
31. Almand, B.; Resser, J. R.; Lindman, B.; Nadaf, S.; Clark, J. I.; Kwon, E. D.; Carbone, D. P.; Gabrilovich, D. I. Clinical Significance of Defective Dendritic Cell Differentiation in Cancer. *Clin. Cancer Res.* **2000**, *6*, 1755–1766.
32. Gross, G.; Eshhar, Z. Endowing T Cells with Antibody Specificity Using Chimeric T Cell Receptors. *FASEB J.* **1992**, *6*, 3370–3378. DOI: 10.1096/fasebj.6.15.1464371.
33. D'Aloia, M. M.; Zizzari, I. G.; Sacchetti, B.; Pierelli, L.; Alimandi, M. CAR-T Cells: The Long and Winding Road to Solid Tumors. *Cell Death Dis.* **2018**, *9*, 282. DOI: 10.1038/s41419-018-0278-6.
34. Moon, J. J.; Huang, B.; Irvine, D. J. Engineering Nano- and Microparticles to Tune Immunity. *Adv. Mater.* **2012**, *24*, 3724–3746. DOI: 10.1002/adma.201200446.
35. Schmid, D.; Park, C. G.; Hartl, C. A.; Subedi, N.; Cartwright, A. N.; Puerto, R. B.; Zheng, Y.; Maiarana, J.; Freeman, G. J.; Wucherpfennig, K. W.; et al. T Cell-Targeting Nanoparticles Focus Delivery of Immunotherapy to Improve Antitumor Immunity. *Nat. Commun.* **2017**, *8*, 1747. DOI: 10.1038/s41467-017-01830-8.
36. Steenblock, E. R.; Fahmy, T. M. A Comprehensive Platform for *Ex Vivo* T-Cell Expansion Based on Biodegradable Polymeric Artificial Antigen-Presenting Cells. *Mol. Ther.* **2008**, *16*, 765–772. DOI: 10.1038/mt.2008.11.
37. Begines, B.; Ortiz, T.; Pérez-Aranda, M.; Martínez, G.; Merinero, M.; Argüelles-Arias, F.; Alcudia, A. Polymeric Nanoparticles for Drug Delivery: Recent Developments and Future Prospects. *Nanomaterials* **2020**, *10*, 1403. DOI: 10.3390/nano10071403.
38. Perica, K.; De León Medero, A.; Durai, M.; Chiu, Y. L.; Bieler, J. G.; Sibener, L.; Niemöller, M.; Assenmacher, M.; Richter, A.; Edidin, M.; et al. Nanoscale Artificial Antigen Presenting Cells for T Cell Immunotherapy. *Nanomedicine* **2014**, *10*, 119–129. DOI: 10.1016/j.nano.2013.06.015.
39. Meyer, R. A.; Sunshine, J. C.; Perica, K.; Kosmides, A. K.; Aje, K.; Schneck, J. P.; Green, J. J. Biodegradable Nanoellipsoidal Artificial Antigen Presenting Cells for Antigen Specific T-Cell Activation. *Small* **2015**, *11*, 1519–1525. DOI: 10.1002/smll.201402369.
40. Kumar, S.; Anselmo, A. C.; Banerjee, A.; Zakrewsky, M.; Mitragotri, S. Shape and Size-Dependent Immune Response to Antigen-Carrying Nanoparticles. *J. Control Release* **2015**, *220*, 141–148. DOI: 10.1016/J.JCONREL.2015.09.069.
41. Sunshine, J. C.; Perica, K.; Schneck, J. P.; Green, J. J. Particle Shape Dependence of $CD8^+$ T Cell Activation by Artificial Antigen Presenting Cells. *Biomaterials* **2014**, *35*, 269–277. DOI: 10.1016/J.BIOMATERIALS.2013.09.050.
42. Glenny, A. T.; Pope, C. G.; Waddington, H.; Wallace, U. Immunological Notes. XVII–XXIV. *J. Pathol.* **1926**, *29*, 31–40. DOI: 10.1002/path.1700290106.
43. Sokolovska, A.; Hem, S. L.; HogenEsch, H. Activation of Dendritic Cells and Induction of $CD4^+$ T Cell Differentiation by Aluminum-Containing Adjuvants. *Vaccine* **2007**, *25*, 4575–4585. DOI: 10.1016/j.vaccine.2007.03.045.
44. Desbien, A. L.; Reed, S. J.; Bailor, H. R.; Cauwelaert, N. D.; Laurance, J. D.; Orr, M. T.; Fox, C. B.; Carter, D.; Reed, S. G.; Duthie, M. S. Squalene Emulsion Potentiates the Adjuvant Activity of the TLR4 Agonist, GLA, via Inflammatory Caspases, IL-18, and IFN-γ. *Eur. J. Immunol.* **2015**, *45*, 407–417. DOI: 10.1002/eji.201444543.

45. Ismaili, J.; Rennesson, J.; Aksoy, E.; Vekemans, J.; Vincart, B.; Amraoui, Z.; Van Laethem, F.; Goldman, M.; Dubois, P. M. Monophosphoryl Lipid A Activates Both Human Dendritic Cells and T Cells. *J. Immunol.* **2002**, *168*, 926–932. DOI: 10.4049/jimmunol.168.2.926.
46. Chu, R. S.; Targoni, O. S.; Krieg, A. M.; Lehmann, P. V.; Harding, C. V. CpG Oligodeoxynucleotides Act as Adjuvants That Switch on T Helper 1 (Th1) Immunity. *J. Exp. Med.* **1997**, *186*, 1623–1631. DOI: 10.1084/jem.186.10.1623.
47. Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, 646–674. DOI: 10.1016/j.cell.2011.02.013.
48. Dunn, G. P.; Bruce, A. T.; Ikeda, H.; Old, L. J.; Schreiber, R. D. Cancer Immunoediting: From Immunosurveillance to Tumor Escape. *Nat. Immunol.* **2002**, *3*, 991–998. DOI: 10.1038/ni1102-991.
49. Curran, M. A.; Montalvo, W.; Yagita, H.; Allison, J. P. PD-1 and CTLA-4 Combination Blockade Expands Infiltrating T Cells and Reduces Regulatory T and Myeloid Cells within B16 Melanoma Tumors. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 4275–4280. DOI: 10.1073/pnas.0915174107.
50. Rajabi, M.; Mousa, S. A. The Role of Angiogenesis in Cancer Treatment. *Biomedicines* **2017**, *5*, 34. DOI: 10.3390/biomedicines5020034.
51. Ferrara, N. Vascular Endothelial Growth Factor as a Target for Anticancer Therapy. *Oncologist* **2004**, *9 Suppl 1*, 2–10. DOI: 10.1634/theoncologist.9-suppl_1-2.
52. Pugh, C. W.; Ratcliffe, P. J. Regulation of Angiogenesis by Hypoxia: Role of the HIF System. *Nat. Med.* **2003**, *9*, 677–684. DOI: 10.1038/nm0603-677.
53. Diaz-Gonzalez, J. A.; Russell, J.; Rouzaut, A.; Gil-Bazo, I.; Montuenga, L. Targeting Hypoxia and Angiogenesis through HIF-1alpha Inhibition. *Cancer Biol. Ther.* **2005**, *4*, 1055–1062. DOI: 10.4161/cbt.4.10.2195.
54. Zhong, H.; Chiles, K.; Feldser, D.; Laughner, E.; Hanrahan, C.; Georgescu, M.-M.; Simons, J. W.; Semenza, G. L. Modulation of Hypoxia-Inducible Factor 1α Expression by the Epidermal Growth Factor/Phosphatidylinositol 3-Kinase/PTEN/AKT/FRAP Pathway in Human Prostate Cancer Cells: Implications for Tumor Angiogenesis and Therapeutics. *Cancer Res.* **2000**, *60*, 1541–1545.
55. Ferrara, N.; Hillan, K. J.; Novotny, W. Bevacizumab (Avastin), a Humanized Anti-VEGF Monoclonal Antibody for Cancer Therapy. *Biochem. Biophys. Res. Commun.* **2005**, *333*, 328–335. DOI: 10.1016/j.bbrc.2005.05.132.
56. Ebewele, R. O. *Polymer Science and Technology*; CRC Press: Boca Raton, FL, **2000**. DOI: 10.1016/0261-3069(95)90127-2.
57. Kamatar, A.; Gunay, G.; Acar, H. Natural and Synthetic Biomaterials for Engineering Multicellular Tumor Spheroids. *Polymers* **2020**, *12*, 2506. DOI: 10.3390/polym12112506.
58. Negut, I.; Dorcioman, G.; Grumezescu, V. Scaffolds for Wound Healing Applications. *Polymers* **2020**, *12*, 2010. DOI: 10.3390/polym12092010.
59. Fan, D.; Staufer, U.; Accardo, A. Engineered 3D Polymer and Hydrogel Microenvironments for Cell Culture Applications. *Bioengineering* **2019**, *6*, 113. DOI: 10.3390/bioengineering6040113.
60. Abbasian, M.; Massoumi, B.; Mohammad-Rezaei, R.; Samadian, H.; Jaymand, M. Scaffolding Polymeric Biomaterials: Are Naturally Occurring Biological Macromolecules More Appropriate for Tissue Engineering? *Int. J. Biol. Macromol.* **2019**, *134*, 673–694. DOI: 10.1016/j.ijbiomac.2019.04.197.
61. Koide, S. S. Chitin-Chitosan: Properties, Benefits and Risks. *Nutr. Res.* **1998**, *18*, 1091–1101. DOI: 10.1016/S0271-5317(98)00091-8.
62. Kafetzopoulos, D.; Martinou, A.; Bouriotis, V. Bioconversion of Chitin to Chitosan: Purification and Characterization of Chitin Deacetylase from *Mucor rouxii*. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2564–2568. DOI: 10.1073/pnas.90.7.2564.
63. Hirano, S.; Zhang, M.; Chung, B. G.; Kim, S. K. The N-Acylation of Chitosan Fibre and the N-Deacetylation of Chitin Fibre and Chitin–Cellulose Blended Fibre at a Solid State. *Carbohydr. Polym.* **2000**, *41*, 175–179. DOI: 10.1016/S0144-8617(99)00081-8.

64. Colvin, J. R. The Biosynthesis of Cellulose. In *Carbohydrates: Structure and Function*; Elsevier: Netherlands, **1980**; pp 543–570.
65. Cannon, R. E.; Anderson, S. M. Biogenesis of Bacterial Cellulose. *Crit. Rev. Microbiol.* **1991**, *17*, 435–447. DOI: 10.3109/10408419109115207.
66. Marchessault, R. H.; Sundararajan, P. R. 2 – Cellulose. In *The Polysaccharides*, Aspinall, G. O. B. T.-T. P., Ed.; Academic Press: Cambridge, MA, **1983**; pp 11–95. DOI: 10.1016/B978-0-12-065602-8.50007-8.
67. Fujino, T.; Itoh, T. Architecture of the Cell Wall of a Green Alga, *Oocystis apiculata*. *Protoplasma* **1994**, *180*, 39–48. DOI: 10.1007/BF01379222.
68. Smidsrød, O.; Skja, G. Alginate as Immobilization Matrix for Cells. *Trends Biotechnol.* **1990**, *8*, 71–78.
69. Haug, A.; Larsen, B.; Smidsrød, O.; Smidsrød, O.; Eriksson, G.; Blinc, R.; Paušak, S.; Ehrenberg, L.; Dumanović, J. Studies on the Sequence of Uronic Acid Residues in Alginic Acid. *Acta Chem. Scand.* **1967**, *21*, 691–704. DOI: 10.3891/acta.chem.scand.21-0691.
70. Gombotz, W. R.; Wee, S. Protein Release from Alginate Matrices. *Adv. Drug Deliv. Rev.* **1998**, *31*, 267–285. DOI: 10.1016/s0169-409x(97)00124-5.
71. Rowley, J. A.; Madlambayan, G.; Mooney, D. J. Alginate Hydrogels as Synthetic Extracellular Matrix Materials. *Biomaterials* **1999**, *20*, 45–53. DOI: 10.1016/s0142-9612(98)00107-0.
72. Fraser, J. R. E.; Laurent, T. C.; Laurent, U. B. G. Hyaluronan: Its Nature, Distribution, Functions and Turnover. *J. Intern. Med.* **1997**, *242*, 27–33. DOI: 10.1046/j.1365-2796.1997.00170.x.
73. Price, R. D.; Berry, M. G.; Navsaria, H. A. Hyaluronic Acid: The Scientific and Clinical Evidence. *J. Plast. Reconstr. Aesthet. Surg.* **2007**, *60*, 1110–1119. DOI: 10.1016/j.bjps.2007.03.005.
74. Ambrosio, L.; Borzacchiello, A.; Netti, P. A.; Nicolais, L. Rheological Study on Hyaluronic Acid and Its Derivative Solutions. *J. Macromol. Sci. Pure Appl. Chem.* **1999**, *36*, 991–1000. DOI: 10.1081/MA-100101578.
75. Laurent, T. C.; Fraser, J. R. E. The Properties and Turnover of Hyaluronan. In *Ciba Found Symp*; Wiley Online Library: Hoboken, NJ, **1986**; Vol. 124, pp 9–29.
76. Chen, W. Y. J. Functions of Hyaluronan in Wound Repair. In *Hyaluronan*; Elsevier: Netherlands, **2002**; pp 147–156.
77. Rayahin, J. E.; Buhrman, J. S.; Zhang, Y.; Koh, T. J.; Gemeinhart, R. A. High and Low Molecular Weight Hyaluronic Acid Differentially Influence Macrophage Activation. *ACS Biomater. Sci. Eng.* **2015**, *1*, 481–493. DOI: 10.1021/acsbiomaterials.5b00181.
78. Vigetti, D.; Karousou, E.; Viola, M.; Deleonibus, S.; De Luca, G.; Passi, A. Hyaluronan: Biosynthesis and Signaling. *Biochim. Biophys. Acta* **2014**, *1840*, 2452–2459. DOI: 10.1016/j.bbagen.2014.02.001.
79. Misra, S.; Hascall, V. C.; Markwald, R. R.; Ghatak, S. Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer. *Front. Immunol.* **2015**, *6*, 201. DOI: 10.3389/fimmu.2015.00201.
80. Senbanjo, L. T.; Chellaiah, M. A. CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. *Front. Cell Dev. Biol.* **2017**, *5*, 18. DOI: 10.3389/fcell.2017.00018.
81. Kuo, J. W.; Prestwich, G. D. Materials of Biological Origin-Materials Analysis and Implant Uses, Comprehensive Biomaterials. In *Comprehensive Biomaterials*, Ducheyne, P., Ed.; Elsevier: Netherlands, **2010**.
82. Valle, D.; Romeo, F. A. Cross-Linked Esters of Hyaluronic Acid. Google Patents, September 18, **1990**.
83. O'Neill, M. A.; Selvendran, R. R.; Morris, V. J. Structure of the Acidic Extracellular Gelling Polysaccharide Produced by *Pseudomonas elodea*. *Carbohydr. Res.* **1983**, *124*, 123–133. DOI: 10.1016/0008-6215(83)88360-8.
84. Chandrasekaran, R.; Millane, R. P.; Arnott, S.; Atkins, E. D. T. The Crystal Structure of Gellan. *Carbohydr. Res.* **1988**, *175*, 1–15. DOI: 10.1016/0008-6215(88)80151-4.

85. Milas, M.; Shi, X.; Rinaudo, M. On the Physicochemical Properties of Gellan Gum. *Biopolymers* **1990**, *30*, 451–464. DOI: 10.1002/bip.360300322.
86. Chauhan, A. S.; Jain, N. K.; Diwan, P. V.; Khopade, A. J. Solubility Enhancement of Indomethacin with Poly (Amidoamine) Dendrimers and Targeting to Inflammatory Regions of Arthritic Rats. *J. Drug Target* **2004**, *12*, 575–583. DOI: 10.1080/10611860400010655.
87. Gellan Gum. In *Thermoreversible Networks: Viscoelastic Properties and Structure of Gels*; Springer Berlin Heidelberg: Berlin; Heidelberg, **1997**; pp 219–235. DOI: 10.1007/BFb0008712.
88. Kersten, G.; Hirschberg, H. Antigen Delivery Systems. *Expert Rev. Vaccines* **2004**, *3*, 453–462. DOI: 10.1586/14760584.3.4.453.
89. Chao, Y.; Xu, L.; Liang, C.; Feng, L.; Xu, J.; Dong, Z.; Tian, L.; Yi, X.; Yang, K.; Liu, Z. Combined Local Immunostimulatory Radioisotope Therapy and Systemic Immune Checkpoint Blockade Imparts Potent Antitumour Responses. *Nat. Biomed. Eng.* **2018**, *2*, 611–621. DOI: 10.1038/s41551-018-0262-6.
90. Shu, G.; Zhu, W.; Jiang, Y.; Li, X.; Pan, J.; Zhang, X.; Zhang, X.; Sun, S.-K. Persistent Luminescence Immune Hydrogel for Photodynamic-Immunotherapy of Tumors *In Vivo*. *Adv. Funct. Mater.* **2021**, *31*, 2104472. DOI: 10.1002/adfm.202104472.
91. Castro, F.; Pinto, M. L.; Pereira, C. L.; Serre, K.; Barbosa, M. A.; Vermaelen, K.; Gärtner, F.; Gonçalves, R. M.; De Wever, O.; Oliveira, M. J. Chitosan/γ-PGA Nanoparticles-Based Immunotherapy as Adjuvant to Radiotherapy in Breast Cancer. *Biomaterials* **2020**, *257*, 120218. DOI: 10.1016/j.biomaterials.2020.120218.
92. Verbeke, C. S.; Gordo, S.; Schubert, D. A.; Lewin, S. A.; Desai, R. M.; Dobbins, J.; Wucherpfennig, K. W.; Mooney, D. J. Multicomponent Injectable Hydrogels for Antigen-Specific Tolerogenic Immune Modulation. *Adv. Healthc. Mater.* **2017**, *6*, 1600773. DOI: 10.1002/adhm.201600773.
93. Verbeke, C. S.; Mooney, D. J. Injectable, Pore-Forming Hydrogels for *In Vivo* Enrichment of Immature Dendritic Cells. *Adv. Healthc. Mater.* **2015**, *4*, 2677–2687. DOI: 10.1002/adhm.201500618.
94. Liu, Y.; Han, Y.-Y.; Lu, S.; Wu, Y.; Li, J.; Sun, X.; Yan, J. Injectable Hydrogel Platform with Biodegradable Dawson-Type Polyoxometalate and R848 for Combinational Photothermal-Immunotherapy of Cancer. *Biomater. Sci.* **2022**, *10*, 1257–1266. DOI: 10.1039/D1BM01835C.
95. Polack, F. P.; Thomas, S. J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J. L.; Pérez Marc, G.; Moreira, E. D.; Zerbini, C.; et al. Safety and Efficacy of the BNT162b2 MRNA Covid-19 Vaccine. *N. Engl. J. Med.* **2020**, *383*, 2603–2615. DOI: 10.1056/NEJMoa2034577.
96. Baden, L. R.; El Sahly, H. M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S. A.; Rouphael, N.; Creech, C. B.; et al. Efficacy and Safety of the MRNA-1273 SARS-CoV-2 Vaccine. *N. Engl. J. Med.* **2021**, *384*, 403–416. DOI: 10.1056/NEJMoa2035389.
97. Corbett, K. S.; Edwards, D. K.; Leist, S. R.; Abiona, O. M.; Boyoglu-Barnum, S.; Gillespie, R. A.; Himansu, S.; Schäfer, A.; Ziwawo, C. T.; DiPiazza, A. T.; et al. SARS-CoV-2 MRNA Vaccine Design Enabled by Prototype Pathogen Preparedness. *Nature* **2020**, *586*, 567–571. DOI: 10.1038/s41586-020-2622-0.
98. Vogel, A. B.; Kanevsky, I.; Che, Y.; Swanson, K. A.; Muik, A.; Vormehr, M.; Kranz, L. M.; Walzer, K. C.; Hein, S.; Güler, A.; et al. BNT162b Vaccines Protect Rhesus Macaques from SARS-CoV-2. *Nature* **2021**, *592*, 283–289. DOI: 10.1038/s41586-021-03275-y.
99. Yan, J.; Chen, R.; Zhang, H.; Bryers, J. D. Injectable Biodegradable Chitosan-Alginate 3D Porous Gel Scaffold for MRNA Vaccine Delivery. *Macromol. Biosci.* **2019**, *19*, e1800242. DOI: 10.1002/mabi.201800242.
100. Duong, H. T. T.; Thambi, T.; Yin, Y.; Kim, S. H.; Nguyen, T. L.; Phan, V. H. G.; Kim, J.; Jeong, J. H.; Lee, D. S. Degradation-Regulated Architecture of Injectable Smart Hydrogels Enhances Humoral Immune Response and Potentiates Antitumor Activity in Human

Lung Carcinoma. *Biomaterials* **2020**, *230*, 119599. DOI: 10.1016/j.biomaterials.2019.119599.

101. Lin, H.; Li, Q.; Wang, O.; Rauch, J.; Harm, B.; Viljoen, H. J.; Zhang, C.; Van Wyk, E.; Zhang, C.; Lei, Y. Automated Expansion of Primary Human T Cells in Scalable and Cell-Friendly Hydrogel Microtubes for Adoptive Immunotherapy. *Adv. Healthc. Mater.* **2018**, *7*, e1701297. DOI: 10.1002/adhm.201701297.
102. Stephan, S. B.; Taber, A. M.; Jileaeva, I.; Pegues, E. P.; Sentman, C. L.; Stephan, M. T. Biopolymer Implants Enhance the Efficacy of Adoptive T-Cell Therapy. *Nat. Biotechnol.* **2015**, *33*, 97–101. DOI: 10.1038/nbt.3104.
103. Ahn, Y. H.; Ren, L.; Kim, S. M.; Seo, S.-H.; Jung, C.-R.; Kim, D. S.; Noh, J.-Y.; Lee, S. Y.; Lee, H.; Cho, M. Y.; et al. A Three-Dimensional Hyaluronic Acid-Based Niche Enhances the Therapeutic Efficacy of Human Natural Killer Cell-Based Cancer Immunotherapy. *Biomaterials* **2020**, *247*, 119960. DOI: 10.1016/j.biomaterials.2020.119960.
104. Smith, T. T.; Moffett, H. F.; Stephan, S. B.; Opel, C. F.; Dumigan, A. G.; Jiang, X.; Pillarisetty, V. G.; Pillai, S. P. S.; Wittrup, K. D.; Stephan, M. T. Biopolymers Codelivering Engineered T Cells and STING Agonists Can Eliminate Heterogeneous Tumors. *J. Clin. Invest.* **2017**, *127*, 2176–2191. DOI: 10.1172/JCI87624.
105. Sinha, A.; Choi, Y.; Nguyen, M. H.; Nguyen, T. L.; Choi, S. W.; Kim, J. A 3D Macroporous Alginate Graphene Scaffold with an Extremely Slow Release of a Loaded Cargo for *In Situ* Long-Term Activation of Dendritic Cells. *Adv. Healthc. Mater.* **2019**, *8*, e1800571. DOI: 10.1002/adhm.201800571.
106. Schwenter, F.; Zarei, S.; Luy, P.; Padrun, V.; Bouche, N.; Lee, J. S.; Mulligan, R. C.; Morel, P.; Mach, N. Cell Encapsulation Technology as a Novel Strategy for Human Anti-Tumor Immunotherapy. *Cancer Gene Ther.* **2011**, *18*, 553–562. DOI: 10.1038/cgt.2011.22.
107. Moran, D. M.; Koniaris, L. G.; Jablonski, E. M.; Cahill, P. A.; Halberstadt, C. R.; McKillop, I. H. Microencapsulation of Engineered Cells to Deliver Sustained High Circulating Levels of Interleukin-6 to Study Hepatocellular Carcinoma Progression. *Cell Transplant.* **2006**, *15*, 785–798. DOI: 10.3727/000000006783981477.
108. Cirone, P.; Bourgeois, J. M.; Shen, F.; Chang, P. L. Combined Immunotherapy and Antiangiogenic Therapy of Cancer with Microencapsulated Cells. *Hum. Gene Ther.* **2004**, *15*, 945–959. DOI: 10.1089/hum.2004.15.945.
109. Huang, X. Q. Depot Cytokines and Chemokines for Antitumor Therapy in a Mouse Model. **2005**.
110. Atik, A. F.; Suryadevara, C. M.; Schweller, R. M.; West, J. L.; Healy, P.; Herndon Ii, J. E.; Congdon, K. L.; Sanchez-Perez, L.; McLendon, R. E.; Archer, G. E.; et al. Hyaluronic Acid Based Low Viscosity Hydrogel as a Novel Carrier for Convection Enhanced Delivery of CAR T Cells. *J. Clin. Neurosci.* **2018**, *56*, 163–168. DOI: 10.1016/j.jocn.2018.06.005.
111. Dautzenberg, H.; Schuldt, U.; Grasnick, G.; Karle, P.; Müller, P.; Löhr, M.; Pelegrin, M.; Piechaczyk, M.; Rombs, K. V.; Günzburg, W. H.; et al. Development of Cellulose Sulfate-Based Polyelectrolyte Complex Microcapsules for Medical Applications. *Ann. N. Y. Acad. Sci.* **1999**, *875*, 46–63. DOI: 10.1111/j.1749-6632.1999.tb08493.x.
112. Abastado, J. S.; Gunzburg, W. H.; Brandtner, E. M. The Diversity of Uses for Cellulose Sulphate Encapsulation. *Bioencapsulation Living Cells Divers. Med. Appl. Bentham Sci.* **2013**, *1*, 70–92.
113. Salmons, B.; Gunzburg, W. H. Release Characteristics of Cellulose Sulphate Capsules and Production of Cytokines from Encapsulated Cells. *Int. J. Pharm.* **2018**, *548*, 15–22. DOI: 10.1016/j.ijpharm.2018.06.040.
114. Yung, C. W.; Bentley, W. E.; Barbari, T. A. Diffusion of Interleukin-2 from Cells Overlaid with Cytocompatible Enzyme-Crosslinked Gelatin Hydrogels. *J. Biomed. Mater. Res. A* **2010**, *95*, 25–32. DOI: 10.1002/jbm.a.32740.
115. Engelke, L.; Winter, G.; Hook, S.; Engert, J. Recent Insights into Cutaneous Immunization: How to Vaccinate via the Skin. *Vaccine* **2015**, *33*, 4663–4674. DOI: 10.1016/j.vaccine.2015.05.012.

116. Chen, M.-C.; Lai, K.-Y.; Ling, M.-H.; Lin, C.-W. Enhancing Immunogenicity of Antigens through Sustained Intradermal Delivery Using Chitosan Microneedles with a Patch-Dissolvable Design. *Acta Biomater.* **2018**, *65*, 66–75. DOI: 10.1016/J.ACTBIO.2017.11.004.
117. Leone, M.; Priester, M. I.; Romeijn, S.; Nejadnik, M. R.; Mönkäre, J.; O'Mahony, C.; Jiskoot, W.; Kersten, G.; Bouwstra, J. A. Hyaluronan-Based Dissolving Microneedles with High Antigen Content for Intradermal Vaccination: Formulation, Physicochemical Characterization and Immunogenicity Assessment. *Eur. J. Pharm. Biopharm.* **2019**, *134*, 49–59. DOI: 10.1016/j.ejpb.2018.11.013.
118. Chiu, Y.-H.; Chen, M.-C.; Wan, S.-W. Sodium Hyaluronate/Chitosan Composite Microneedles as a Single-Dose Intradermal Immunization System. *Biomacromolecules* **2018**, *19*, 2278–2285. DOI: 10.1021/acs.biomac.8b00441.
119. Ye, Y.; Wang, C.; Zhang, X.; Hu, Q.; Zhang, Y.; Liu, Q.; Wen, D.; Milligan, J.; Bellotti, A.; Huang, L.; et al. A Melanin-Mediated Cancer Immunotherapy Patch. *Sci. Immunol.* **2017**, *2*. DOI: 10.1126/sciimmunol.aan5692.
120. Kim, H.; Seong, K.-Y.; Lee, J. H.; Park, W.; Yang, S. Y.; Hahn, S. K. Biodegradable Microneedle Patch Delivering Antigenic Peptide-Hyaluronate Conjugate for Cancer Immunotherapy. *ACS Biomater. Sci. Eng.* **2019**, *5*, 5150–5158. DOI: 10.1021/acsbiomaterials.9b00961.
121. Puigmal, N.; Dosta, P.; Solhjou, Z.; Yatim, K.; Ramírez, C.; Choi, J. Y.; Alhaddad, J. B.; Cosme, A. P.; Azzi, J.; Artzi, N. Microneedle-Based Local Delivery of CCL22 and IL-2 Enriches Treg Homing to the Skin Allograft and Enables Temporal Monitoring of Immunotherapy Efficacy. *Adv. Funct. Mater.* **2021**, *31*, 2100128. DOI: 10.1002/adfm.202100128.
122. Wang, C.; Ye, Y.; Hochu, G. M.; Sadeghifar, H.; Gu, Z. Enhanced Cancer Immunotherapy by Microneedle Patch-Assisted Delivery of Anti-PD1 Antibody. *Nano Lett.* **2016**, *16*, 2334–2340. DOI: 10.1021/acs.nanolett.5b05030.
123. Yang, P.; Lu, C.; Qin, W.; Chen, M.; Quan, G.; Liu, H.; Wang, L.; Bai, X.; Pan, X.; Wu, C. Construction of a Core-Shell Microneedle System to Achieve Targeted Co-delivery of Checkpoint Inhibitors for Melanoma Immunotherapy. *Acta Biomater.* **2020**, *104*, 147–157. DOI: 10.1016/j.actbio.2019.12.037.
124. Joo, S.-H.; Kim, J.; Hong, J.; Fakhraei Lahiji, S.; Kim, Y.-H. Dissolvable Self-Locking Microneedle Patches Integrated with Immunomodulators for Cancer Immunotherapy. *Adv. Mater.* **2023**, *35*, 2209966. DOI: 10.1002/adma.202209966.
125. Tsai, W.; Tsai, H.; Wong, Y.; Hong, J.; Chang, S.; Lee, M. Preparation and Characterization of Gellan Gum/Glucosamine/Clioquinol Film as Oral Cancer Treatment Patch. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2018**, *82*, 317–322. DOI: 10.1016/j.msec.2017.05.040.
126. Engelke, L.; Winter, G.; Engert, J. Application of Water-Soluble Polyvinyl Alcohol-Based Film Patches on Laser Microporated Skin Facilitates Intradermal Macromolecule and Nanoparticle Delivery. *Eur. J. Pharm. Biopharm.* **2018**, *128*, 119–130. DOI: 10.1016/j.ejpb.2018.04.008.
127. Abioye, A. O.; Issah, S.; Kola-Mustapha, A. T. *Ex Vivo* Skin Permeation and Retention Studies on Chitosan–Ibuprofen–Gellan Ternary Nanogel Prepared by *In Situ* Ionic Gelation Technique—A Tool for Controlled Transdermal Delivery of Ibuprofen. *Int. J. Pharm.* **2015**, *490*, 112–130. DOI: 10.1016/j.ijpharm.2015.05.030.
128. Bigucci, F.; Abruzzo, A.; Saladini, B.; Gallucci, M. C.; Cerchiara, T.; Luppi, B. Development and Characterization of Chitosan/Hyaluronan Film for Transdermal Delivery of Thiocolchicoside. *Carbohydr. Polym.* **2015**, *130*, 32–40. DOI: 10.1016/j.carbpol.2015.04.067.
129. Morad, H.; Jahanshahi, M.; Akbari, J.; Saeedi, M.; Gill, P.; Enayatifard, R. Novel Topical and Transdermal Delivery of Colchicine with Chitosan Based Biocomposite Nanofiberous System; Formulation, Optimization, Characterization, *Ex Vivo* Skin Deposition/Permeation, and Anti-Melanoma Evaluation. *Mater. Chem. Phys.* **2021**, *263*, 124381. DOI: 10.1016/j.matchemphys.2021.124381.

130. Li, Y.; Li, X.; Doughty, A.; West, C.; Wang, L.; Zhou, F.; Nordquist, R. E.; Chen, W. R. Phototherapy Using Immunologically Modified Carbon Nanotubes to Potentiate Checkpoint Blockade for Metastatic Breast Cancer. *Nanomedicine* **2019**, *18*, 44–53. DOI: 10.1016/j.nano.2019.02.009.
131. Saha, L. C.; Nag, O. K.; Doughty, A.; Liu, H.; Chen, W. R. An Immunologically Modified Nanosystem Based on Noncovalent Binding Between Single-Walled Carbon Nanotubes and Glycated Chitosan. *Technol. Cancer Res. Treat.* **2018**, *17*, 1533033818802313. DOI: 10.1177/1533033818802313.
132. Lin, L.; He, J.; Li, J.; Xu, Y.; Li, J.; Wu, Y. Chitosan Nanoparticles Strengthen Vγ9Vδ2 T-Cell Cytotoxicity Through Upregulation Of Killing Molecules And Cytoskeleton Polarization. *Int. J. Nanomedicine* **2019**, *14*, 9325–9336. DOI: 10.2147/IJN.S212898.
133. Wusiman, A.; Gu, P.; Liu, Z.; Xu, S.; Zhang, Y.; Hu, Y.; Liu, J.; Wang, D.; Huang, X. Cationic Polymer Modified PLGA Nanoparticles Encapsulating Alhagi Honey Polysaccharides as a Vaccine Delivery System for Ovalbumin to Improve Immune Responses. *Int. J. Nanomedicine* **2019**, *14*, 3221–3234. DOI: 10.2147/IJN.S203072.
134. Tahamtan, A.; Barati, M.; Tabarraei, A.; Mohebbi, S. R.; Shirian, S.; Gorji, A.; Ghaemi, A. Antitumor Immunity Induced by Genetic Immunization with Chitosan Nanoparticle Formulated Adjuvanted for HPV-16 E7 DNA Vaccine. *Iran. J. Immunol.* **2018**, *15*, 269–280. DOI: 10.22034/IJI.2018.39396.
135. Hoemann, C. D.; Fong, D. 3 – Immunological Responses to Chitosan for Biomedical Applications. In *Chitosan Based Biomaterials*, Jennings, J. A., Bumgardner, J. D. B. T.-C. B. B., Eds.; Woodhead Publishing: United Kingdom, **2017**; Vol. 1, pp 45–79. DOI: 10.1016/B978-0-08-100230-8.00003-0.
136. Zaharoff, D. A.; Hance, K. W.; Rogers, C. J.; Schlom, J.; Greiner, J. W. Intratumoral Immunotherapy of Established Solid Tumors with Chitosan/IL-12. *J. Immunother.* **2010**, *33*, 697–705. DOI: 10.1097/CJI.0b013e3181eb826d.
137. Choi, J. J.; Le, Q.-V.; Kim, D.; Kim, Y. B.; Shim, G.; Oh, Y.-K. High Molecular Weight Chitosan-Complexed RNA Nanoadjuvant for Effective Cancer Immunotherapy. *Pharmaceutics* **2019**, *11*, 680. DOI: 10.3390/pharmaceutics11120680.
138. Maiyo, F.; Singh, M. Folate-Targeted MRNA Delivery Using Chitosan-Functionalized Selenium Nanoparticles: Potential in Cancer Immunotherapy. *Pharmaceuticals* **2019**, *12* (4),164. DOI: 10.3390/ph12040164.
139. Masjedi, A.; Hassannia, H.; Atyabi, F.; Rastegari, A.; Hojjat-Farsangi, M.; Namdar, A.; Soleimanpour, H.; Azizi, G.; Nikkhoo, A.; Ghalamfarsa, G.; et al. Downregulation of A2AR by SiRNA Loaded PEG-Chitosan-Lactate Nanoparticles Restores the T Cell Mediated Anti-Tumor Responses through Blockage of PKA/CREB Signaling Pathway. *Int. J. Biol. Macromol.* **2019**, *133*, 436–445. DOI: 10.1016/j.ijbiomac.2019.03.223.
140. Arab, S.; Kheshtchin, N.; Ajami, M.; Ashurpoor, M.; Safvati, A.; Namdar, A.; Mirzaei, R.; Mousavi Niri, N.; Jadidi-Niaragh, F.; Ghahremani, M. H.; et al. Increased Efficacy of a Dendritic Cell-Based Therapeutic Cancer Vaccine with Adenosine Receptor Antagonist and CD73 Inhibitor. *Tumour Biol.* **2017**, *39*, 1010428317695021. DOI: 10.1177/1010428317695021.
141. Dong, Z.; Kang, Y.; Yuan, Q.; Luo, M.; Gu, Z. H(2)O(2)-Responsive Nanoparticle Based on the Supramolecular Self-Assemble of Cyclodextrin. *Front. Pharmacol.* **2018**, *9*, 552. DOI: 10.3389/fphar.2018.00552.
142. Lisanti, M. P.; Martinez-Outschoorn, U. E.; Lin, Z.; Pavlides, S.; Whitaker-Menezes, D.; Pestell, R. G.; Howell, A.; Sotgia, F. Hydrogen Peroxide Fuels Aging, Inflammation, Cancer Metabolism and Metastasis: The Seed and Soil Also Needs "Fertilizer". *Cell Cycle* **2011**, *10*, 2440–2449. DOI: 10.4161/cc.10.15.16870.
143. Cai, Z.; Xin, F.; Wei, Z.; Wu, M.; Lin, X.; Du, X.; Chen, G.; Zhang, D.; Zhang, Z.; Liu, X.; et al. Photodynamic Therapy Combined with Antihypoxic Signaling and CpG Adjuvant as an *In Situ* Tumor Vaccine Based on Metal-Organic Framework Nanoparticles to Boost Cancer Immunotherapy. *Adv. Healthc. Mater.* **2020**, *9*, e1900996. DOI: 10.1002/adhm.201900996.

144. Sun, W.; Du, Y.; Liang, X.; Yu, C.; Fang, J.; Lu, W.; Guo, X.; Tian, J.; Jin, Y.; Zheng, J. Synergistic Triple-Combination Therapy with Hyaluronic Acid-Shelled PPy/CPT Nanoparticles Results in Tumor Regression and Prevents Tumor Recurrence and Metastasis in 4T1 Breast Cancer. *Biomaterials* **2019**, *217*, 119264. DOI: 10.1016/j.biomaterials.2019.119264.
145. Bartheldyová, E.; Effenberg, R.; Mašek, J.; Procházka, L.; Knötigová, P. T.; Kulich, P.; Hubatka, F.; Velínská, K.; Zelníčková, J.; Zouharová, D.; et al. Hyaluronic Acid Surface Modified Liposomes Prepared via Orthogonal Aminoxy Coupling: Synthesis of Nontoxic Aminoxylipids Based on Symmetrically α-Branched Fatty Acids, Preparation of Liposomes by Microfluidic Mixing, and Targeting to Cancer Cells Expressi. *Bioconjug. Chem.* **2018**, *29*, 2343–2356. DOI: 10.1021/acs.bioconjchem.8b00311.
146. Lee, H.; Park, H.; Yu, H. S.; Na, K.; Oh, K. T.; Lee, E. S. Dendritic Cell-Targeted PH-Responsive Extracellular Vesicles for Anticancer Vaccination. *Pharmaceutics* **2019**, *11*, 54. DOI: 10.3390/pharmaceutics11020054.
147. He, M.; Huang, L.; Hou, X.; Zhong, C.; Bachir, Z. A.; Lan, M.; Chen, R.; Gao, F. Efficient Ovalbumin Delivery Using a Novel Multifunctional Micellar Platform for Targeted Melanoma Immunotherapy. *Int. J. Pharm.* **2019**, *560*, 1–10. DOI: 10.1016/j.ijpharm.2019.01.027.
148. Li, C.-X.; Zhang, Y.; Dong, X.; Zhang, L.; Liu, M.-D.; Li, B.; Zhang, M.-K.; Feng, J.; Zhang, X.-Z. Artificially Reprogrammed Macrophages as Tumor-Tropic Immunosuppression-Resistant Biologics to Realize Therapeutics Production and Immune Activation. *Adv. Mater.* **2019**, *31*, e1807211. DOI: 10.1002/adma.201807211.
149. Thiery, J.; Keefe, D.; Boulant, S.; Boucrot, E.; Walch, M.; Martinvalet, D.; Goping, I. S.; Bleackley, R. C.; Kirchhausen, T.; Lieberman, J. Perforin Pores in the Endosomal Membrane Trigger the Release of Endocytosed Granzyme B into the Cytosol of Target Cells. *Nat. Immunol.* **2011**, *12*, 770–777. DOI: 10.1038/ni.2050.
150. Qian, X.; Shi, Z.; Qi, H.; Zhao, M.; Huang, K.; Han, D.; Zhou, J.; Liu, C.; Liu, Y.; Lu, Y.; et al. A Novel Granzyme B Nanoparticle Delivery System Simulates Immune Cell Functions for Suppression of Solid Tumors. *Theranostics* **2019**, *9*, 7616–7627. DOI: 10.7150/thno.35900.
151. Li, Q.; Zhang, D.; Zhang, J.; Jiang, Y.; Song, A.; Li, Z.; Luan, Y. A Three-in-One Immunotherapy Nanoweapon via Cascade-Amplifying Cancer-Immunity Cycle against Tumor Metastasis, Relapse, and Postsurgical Regrowth. *Nano Lett.* **2019**, *19*, 6647–6657. DOI: 10.1021/acs.nanolett.9b02923.
152. Lu, R.; Groer, C.; Kleindl, P. A.; Moulder, K. R.; Huang, A.; Hunt, J. R.; Cai, S.; Aires, D. J.; Berkland, C.; Forrest, M. L. Formulation and Preclinical Evaluation of a Toll-Like Receptor 7/8 Agonist as an Anti-Tumoral Immunomodulator. *J. Control Release* **2019**, *306*, 165–176. DOI: 10.1016/j.jconrel.2019.06.003.
153. Gao, F.; Zhang, C.; Qiu, W.-X.; Dong, X.; Zheng, D.-W.; Wu, W.; Zhang, X.-Z. PD-1 Blockade for Improving the Antitumor Efficiency of Polymer-Doxorubicin Nanoprodrug. *Small* **2018**, *14*, e1802403. DOI: 10.1002/smll.201802403.
154. Salazar, A. Method for Preparation of Poly-Iclc and Uses Thereof. WO2005102278A1, July 1, **2003**.
155. Hammerich, L.; Marron, T. U.; Upadhyay, R.; Svensson-Arvelund, J.; Dhainaut, M.; Hussein, S.; Zhan, Y.; Ostrowski, D.; Yellin, M.; Marsh, H.; et al. Systemic Clinical Tumor Regressions and Potentiation of PD1 Blockade with *In Situ* Vaccination. *Nat. Med.* **2019**, *25*, 814–824. DOI: 10.1038/s41591-019-0410-x.
156. Chapman, N. M.; Gottschalk, S.; Chi, H. Preventing Ubiquitination Improves CAR T Cell Therapy via 'CAR Merry-Go-Around'. *Immunity* **2020**, *53*, 243–245. DOI: 10.1016/j.immuni.2020.07.023.
157. Li, W.; Qiu, S.; Chen, J.; Jiang, S.; Chen, W.; Jiang, J.; Wang, F.; Si, W.; Shu, Y.; Wei, P.; et al. Chimeric Antigen Receptor Designed to Prevent Ubiquitination and Downregulation Showed Durable Antitumor Efficacy. *Immunity* **2020**, *53*, 456–470.e6. DOI: 10.1016/j.immuni.2020.07.011.

158. Tie, Y.; Tang, F.; Wei, Y.; Wei, X. Immunosuppressive Cells in Cancer: Mechanisms and Potential Therapeutic Targets. *J. Hematol. Oncol.* **2022**, *15*, 61. DOI: 10.1186/s13045-022-01282-8.
159. Li, X.; Wang, S.; Mu, W.; Barry, J.; Han, A.; Carpenter, R. L.; Jiang, B.-H.; Peiper, S. C.; Mahoney, M. G.; Aplin, A. E.; et al. Reactive Oxygen Species Reprogram Macrophages to Suppress Antitumor Immune Response through the Exosomal MiR-155-5p/PD-L1 Pathway. *J. Exp. Clin. Cancer Res.* **2022**, *41*, 41. DOI: 10.1186/s13046-022-02244-1.
160. Muth, S.; Klaric, A.; Radsak, M.; Schild, H.; Probst, H. C. CD27 Expression on Treg Cells Limits Immune Responses against Tumors. *J. Mol. Med.* **2022**, *100*, 439–449. DOI: 10.1007/s00109-021-02116-9.
161. Assi, H. H.; Wong, C.; Tipton, K. A.; Mei, L.; Wong, K.; Razo, J.; Chan, C.; Howng, B.; Sagert, J.; Krimm, M.; et al. Conditional PD-1/PD-L1 Probody Therapeutics Induce Comparable Antitumor Immunity but Reduced Systemic Toxicity Compared with Traditional Anti-PD-1/PD-L1 Agents. *Cancer Immunol. Res.* **2021**, *9*, 1451–1464. DOI: 10.1158/2326-6066.CIR-21-0031.
162. Boustany, L. M.; LaPorte, S. L.; Wong, L.; White, C.; Vinod, V.; Shen, J.; Yu, W.; Koditek, D.; Winter, M. B.; Moore, S. J.; et al. A Probody T Cell-Engaging Bispecific Antibody Targeting EGFR and CD3 Inhibits Colon Cancer Growth with Limited Toxicity. *Cancer Res.* **2022**, *82*, 4288–4298. DOI: 10.1158/0008-5472.CAN-21-2483.
163. Naing, A.; Thistlethwaite, F.; De Vries, E. G. E.; Eskens, F. A. L. M.; Uboha, N.; Ott, P. A.; LoRusso, P.; Garcia-Corbacho, J.; Boni, V.; Bendell, J.; et al. CX-072 (Pacmilimab), a Probody (®) PD-L1 Inhibitor, in Advanced or Recurrent Solid Tumors (PROCLAIM-CX-072): An Open-Label Dose-Finding and First-in-Human Study. *J. Immunother. Cancer* **2021**, *9*, e002447. DOI: 10.1136/jitc-2021-002447.
164. Han, X.; Bryson, P. D.; Zhao, Y.; Cinay, G. E.; Li, S.; Guo, Y.; Siriwon, N.; Wang, P. Masked Chimeric Antigen Receptor for Tumor-Specific Activation. *Mol. Ther.* **2017**, *25*, 274–284. DOI: 10.1016/j.ymthe.2016.10.011.
165. Juillerat, A.; Marechal, A.; Filhol, J. M.; Valogne, Y.; Valton, J.; Duclert, A.; Duchateau, P.; Poirot, L. An Oxygen Sensitive Self-Decision Making Engineered CAR T-Cell. *Sci. Rep.* **2017**, *7*, 39833. DOI: 10.1038/srep39833.
166. Alkhader, E.; Billa, N.; Roberts, C. J. Mucoadhesive Chitosan-Pectinate Nanoparticles for the Delivery of Curcumin to the Colon. *AAPS PharmSciTech* **2017**, *18*, 1009–1018. DOI: 10.1208/s12249-016-0623-y.
167. Saeed, R. M.; Dmour, I.; Taha, M. O. Stable Chitosan-Based Nanoparticles Using Polyphosphoric Acid or Hexametaphosphate for Tandem Ionotropic/Covalent Crosslinking and Subsequent Investigation as Novel Vehicles for Drug Delivery. *Front. Bioeng. Biotechnol.* **2020**, *8*, 4. DOI: 10.3389/fbioe.2020.00004.
168. Espinosa-Cano, E.; Huerta-Madronal, M.; Camara-Sanchez, P.; Seras-Franzoso, J.; Schwartz, S. Jr.; Abasolo, I.; San Román, J.; Aguilar, M. R. Hyaluronic Acid (HA)-Coated Naproxen-Nanoparticles Selectively Target Breast Cancer Stem Cells through COX-Independent Pathways. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2021**, *124*, 112024. DOI: 10.1016/j.msec.2021.112024.
169. Festas, A. J.; Ramos, A.; Davim, J. P. Medical Devices Biomaterials – A Review. *Proc. Inst. Mech. Eng. Part L. J. Mater. Des. Appl.* **2020**, *234*, 218–228. DOI: 10.1177/1464420719882458.
170. Zhu, L.; Ge, F.; Yang, L.; Li, W.; Wei, S.; Tao, Y.; Du, G. Alginate Particles with Ovalbumin (OVA) Peptide Can Serve as a Carrier and Adjuvant for Immune Therapy in B16-OVA Cancer Model. *Med. Sci. Monit. Basic Res.* **2017**, *23*, 166–172. DOI: 10.12659/MSMBR.901576.
171. Kim, S.; Heo, R.; Song, S. H.; Song, K.-H.; Shin, J. M.; Oh, S. J.; Lee, H.-J.; Chung, J. E.; Park, J. H.; Kim, T. W. PD-L1 SiRNA-Hyaluronic Acid Conjugate for Dual-Targeted Cancer Immunotherapy. *J. Control Release* **2022**, *346*, 226–239. DOI: 10.1016/j.jconrel.2022.04.023.
172. Dalla Pietà, A.; Carpanese, D.; Grigoletto, A.; Tosi, A.; Dalla Santa, S.; Pedersen, G. K.; Christensen, D.; Meléndez-Alafort, L.; Barbieri, V.; De Benedictis, P.; et al. Hyaluronan Is

a Natural and Effective Immunological Adjuvant for Protein-Based Vaccines. *Cell Mol. Immunol.* **2021**, *18*, 1197–1210. DOI: 10.1038/s41423-021-00667-y.

173. Lee, S.-J.; Lee, H.-S.; Hwang, Y.-H.; Kim, J.-J.; Kang, K.-Y.; Kim, S. J.; Kim, H. K.; Kim, J. D.; Jeong, D. H.; Paik, M.-J.; et al. Enhanced Anti-Tumor Immunotherapy by Dissolving Microneedle Patch Loaded Ovalbumin. *PLoS One* **2019**, *14*, e0220382. DOI: 10.1371/journal.pone.0220382.
174. AbdelAllah, N. H.; Gaber, Y.; AbdelGhani, S.; Rashed, M. E.; Azmy, A. F. Chitosan and Alginate Salt as Biomaterials Are Potential Natural Adjuvants for Killed Cholera Vaccine. *J. Biomed. Mater. Res. A* **2021**, *109*, 2462–2470. DOI: 10.1002/jbm.a.37240.
175. Norpi, A. S. M.; Nordin, M. L.; Ahmad, N.; Katas, H.; Fuaad, A. A.-H. A.; Sukri, A.; Marasini, N.; Azmi, F. New Modular Platform Based on Multi-Adjuvanted Amphiphilic Chitosan Nanoparticles for Efficient Lipopeptide Vaccine Delivery against Group A Streptococcus. *Asian J. Pharm. Sci.* **2022**, *17*, 435–446. DOI: 10.1016/j.ajps.2022.04.002.
176. Catania, G.; Rodella, G.; Vanvarenberg, K.; Préat, V.; Malfanti, A. Combination of Hyaluronic Acid Conjugates with Immunogenic Cell Death Inducer and CpG for Glioblastoma Local Chemo-Immunotherapy Elicits an Immune Response and Induces Long-Term Survival. *Biomaterials* **2023**, *294*, 122006. DOI: 10.1016/j.biomaterials.2023.122006.
177. Cao, F.; Yan, M.; Liu, Y.; Liu, L.; Ma, G. Photothermally Controlled MHC Class I Restricted $CD8^+$ T-Cell Responses Elicited by Hyaluronic Acid Decorated Gold Nanoparticles as a Vaccine for Cancer Immunotherapy. *Adv. Healthc. Mater.* **2018**, *7*, 1701439. DOI: 10.1002/adhm.201701439.
178. Wu, C.; Xu, J.; Xie, Z.; Huang, H.; Li, N.; Wei, X.; Li, T.; Yang, H.; Li, S.; Qin, X.; et al. Light-Responsive Hyaluronic Acid Nanomicelles Co-Loaded with an IDO Inhibitor Focus Targeted Photoimmunotherapy against "Immune Cold" Cancer. *Biomater. Sci.* **2021**, *9*, 8019–8031. DOI: 10.1039/d1bm01409a.
179. Shan, G.; Meihe, L.; Minchao, K.; Rui, Z.; Xiaopeng, W.; Guangjian, Z.; Jin, Z. Identification and Validation of Osteopontin and Receptor for Hyaluronic Acid-Mediated Motility (RHAMM, CD168) for Potential Immunotherapeutic Significance of in Lung Squamous Cell Carcinoma. *Int. Immunopharmacol.* **2022**, *107*, 108715. DOI: 10.1016/j.intimp.2022.108715.
180. Claverie, M.; McReynolds, C.; Petitpas, A.; Thomas, M.; Fernandes, S. C. M. Marine-Derived Polymeric Materials and Biomimetics: An Overview. *Polymers* **2020**, *12*, 1002. DOI: 10.3390/polym12051002.
181. Saeed, A. F. U. H.; Su, J.; Ouyang, S. Marine-Derived Drugs: Recent Advances in Cancer Therapy and Immune Signaling. *Biomed. Pharmacother.* **2021**, *134*, 111091. DOI: 10.1016/j.biopha.2020.111091.