Immunomodulatory Biomaterials for Cancer Immunotherapy

Larry Donnell Stokes Jr

Follow this and additional works at: https://egrove.olemiss.edu/hon_thesis

Part of the Biomaterials Commons

Recommended Citation
https://egrove.olemiss.edu/hon_thesis/1767

This Undergraduate Thesis is brought to you for free and open access by the Honors College (Sally McDonnell Barksdale Honors College) at eGrove. It has been accepted for inclusion in Honors Theses by an authorized administrator of eGrove. For more information, please contact egrove@olemiss.edu.
Immunomodulatory Biomaterials for Cancer Immunotherapy

By
Larry D. Stokes, Jr.

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford, MS
May 2021

Approved By
Advisor: Dr. Thomas Werfel
Reader: Dr. Adam Smith
Reader: Dr. Mirela Ovreiu
Dedication

This thesis is dedicated to everyone who guided and encouraged me throughout my time at the University of Mississippi. This work will serve as proof that I was able to succeed despite the obstacles I have faced along the journey, but this is only the beginning.
Acknowledgements

I would like to thank my research advisor for helping me over the summer. I would like to thank my readers for taking the time to help me create this work. I would also like to thank the Ronald E. McNair program for giving me the opportunity and guidance in researching this topic over the summer in 2020.
Abstract

Cancer immunotherapy has become an effective treatment in the toolbox of oncologists. Immunotherapy offers a less toxic alternative to standard cancer treatments such as chemotherapy and can have prolonged curative effects to decrease cancer recurrence. Today, many drugs and biological agents have been developed that target the immune system and elicit an antitumor/cancer response. These agents are known collectively as cancer immunotherapies. While immunotherapies have radically improved treatment outcomes for many cancer patients, there are drawbacks to using these treatments. Immunotherapy treatments have poor clinical responses in patients with tumors that lack immunogenicity. Some of the treatments also pose a risk to induce systemic toxicity when used at high doses and risks of autoimmunity are essentially inherent. To mitigate these shortcomings of immunotherapies, biomaterials can be used as a delivery vehicle to alter the pharmacokinetics, biodistribution, and control release of therapeutic agents targeting the immune system. This review article outlines the general design considerations of various biomaterials and their applications in cancer immunomodulation. Many studies show promising results in murine tumor models with potential for translation to human disease, but further research – via rigorous clinical trials – is needed to assess the effectiveness of immunomodulatory biomaterials in cancer patients.
# Table of Contents

List of Figures .............................................................................................................. VIII

List of Abbreviations .................................................................................................... IX

Introduction ...................................................................................................................... 1

Local Immunomodulatory Biomaterials ........................................................................ 6

Hydrogels ......................................................................................................................... 7

Design Considerations for Effective Drug Delivery ...................................................... 8

Synthetic and Natural Polymers .................................................................................... 11

Stimuli-Responsive “Smart” Hydrogels .......................................................................... 13

Scaffolds ............................................................................................................................. 15

Injectable vs Surgical ...................................................................................................... 16

Design Parameters ......................................................................................................... 19

Microparticles ................................................................................................................ 22

Types of Microparticles and Their Applications ........................................................... 24

Design Considerations ................................................................................................... 30

Systemic Immunomodulatory Biomaterials .................................................................. 32

Nanoparticles .................................................................................................................. 33

Design Considerations ................................................................................................... 37
Types of Nanoparticles and Their Applications…………………………40

Drug Conjugates…………………………………………………………………47

Polymer-Drug Conjugates: Design Considerations……………………..48

Antibody-Drug Conjugates: Design Considerations…………………...51

Conclusion……………………………………………………………………………….53

References……………………………………………………………………………….55

VII
List of Figures

Table 1  US Food and Drug Administration Approved Cancer Immunotherapies  2
Figure 1  Representative Figure of Local and Systemic Drug Delivery  5
Figure 2  Relationship Between the Cross-Linking Density and Physical Properties of the Hydrogel  10
Figure 3  Prophylactic Capability of Nanoplex DNA Vaccine Against Murine B16/OVA Lung Melanoma  13
Figure 4  Process Schematic of Infection-Mimicking Scaffold Design by Ali  16
Figure 5  Prophylactic Cancer Vaccine Study Using Injectable, Spontaneously Forming MSR Scaffolds  19
Figure 6  Various Release Mechanisms of Microparticle Systems  24
Figure 7  Murine B16F10 Melanoma Prophylactic Study Using Fe₃O₄/T-MPs-CpG/Lipo Vaccine  26
Figure 8  Therapeutic Efficacy of AntiCD40 and AntiCTLA-4 Microparticle Formulation Against MC-38 Tumor Cells  29
Figure 9  Schematic of Cell Targeted Delivery of Immunotherapeutic Agents Using Nanoparticles  36
Figure 10 Therapeutic Efficacy of Immunoliposome IL-2-Fc/αCD137 Therapy  42
Figure 11 Therapeutic Effect of Targeted Delivery of SD-208 Using Anti-PD-1 Tagged Nanoparticles  44
Figure 12 Therapeutic Effect of Targeted Delivery of R848 and Sensitization of Tumors to PD-1 Blockade  45
Figure 13 In vivo Anti-Tumor Efficacy of P(L-SS-PTX), a Polymer-Paclitaxel Drug Conjugate  50
Figure 14 Key Design Components of Antibody-Drug Conjugates  52
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>PD</td>
<td>Programmed cell death protein</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>MPS</td>
<td>Mononuclear phagocyte system</td>
</tr>
<tr>
<td>TME</td>
<td>Tumor microenvironment</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>HA-PCLA</td>
<td>Poly (ε-caprolactone-co-lactide) ester-functionalized hyaluronic acid</td>
</tr>
<tr>
<td>HA</td>
<td>Hyaluronic acid</td>
</tr>
<tr>
<td>PELG</td>
<td>Poly (γ-ethyl-L-glutamate)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly (ethylene glycol)</td>
</tr>
<tr>
<td>PLG</td>
<td>Poly-lactide-co-glycolide</td>
</tr>
<tr>
<td>mPEG-b-(PLL-DTPA)</td>
<td>3,3’-dithiodipropionic acid functionalized methoxy poly(ethylene glycol)-b-poly(lysine)</td>
</tr>
<tr>
<td>CpG-ODN</td>
<td>Unmethylated cytosine-phosphate-guanine oligonucleotide</td>
</tr>
<tr>
<td>STING</td>
<td>Stimulator of Interferon Genes</td>
</tr>
<tr>
<td>MSR</td>
<td>Mesoporous silica rods</td>
</tr>
<tr>
<td>OVA</td>
<td>Ovalbumin</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Th_{1/2}</td>
<td>Helper T cell subtype 1/2</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>RGD/RDG</td>
<td>Integrin-binding ligand of Arg-Gly-Asp</td>
</tr>
<tr>
<td>T-MPs</td>
<td>Tumor microparticles</td>
</tr>
<tr>
<td>FITC-BSA</td>
<td>Bovine-fluorescein isothiocyanate</td>
</tr>
<tr>
<td>CTLA</td>
<td>Cytotoxic T lymphocyte antigen</td>
</tr>
<tr>
<td>pLHMGA</td>
<td>Poly (lactic-co-hydroxymethyl-glycolic acid)</td>
</tr>
<tr>
<td>IFA</td>
<td>Incomplete Freund’s adjuvant</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>EPR</td>
<td>Enhanced permeability and retention effect</td>
</tr>
<tr>
<td>DOTAP</td>
<td>Dioleoylphosphatidylic acid</td>
</tr>
<tr>
<td>PTX</td>
<td>Paclitaxel</td>
</tr>
</tbody>
</table>
Introduction

Immunotherapy has become a promising tool in the arsenal of cancer treatment that utilizes the patient’s immune system to elicit robust, long-lasting anticancer responses. Since the FDA approval of the use of IFN-α, a cytokine, in 1986 to treat leukemia, cancer immunotherapy has made significant progress in the development of effective immunomodulatory treatments with promising results in the fight against cancer \(^{[1-3]}\). These treatments include checkpoint inhibitors, CAR T-cell therapy, cytokines, cancer vaccines, monoclonal antibodies, and other immunomodulators that boost the cancer immune response. Several immunomodulatory drugs and agents have been approved by the FDA since 1986 (Table 1). Even though these treatments have proven effective, there are shortcoming as well \(^{[4]}\). In particular, immunotherapy treatments using cytokines, cancer vaccines, and immune checkpoint inhibitors have proven to be less effective in some patients. One of the main issues in these non-responsive patients is a lack of immunogenicity in tumors. Many immunotherapies elicit a change in key immune cells such as dendritic cells (DC) or T-cells; however, tumors with poor immunogenicity, or “cold” tumors, lack many of these cells or possess immune cells that oppose activation of the immune system. Another issue that arises is the risk of systemic toxicity. Therapies such as Interleukin-2 (IL-2) or programmed cell death protein 1 (PD-1) inhibitors can have adverse off target effects and become toxic at high doses \(^{[5-6]}\). Due to this toxicity, only small doses of the drugs can be used, so treatment must be focused to ensure the maximum effectiveness of the drug. Additionally, using immunomodulatory agents can lead to the
development of an autoimmune response. In the case of cancer vaccines, treatments can be ineffective due to a low number of transfections in DCs thus producing a weak anticancer immune response.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Approved cancers</th>
<th>Year of first approval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Checkpoint inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>CTLA4 mAb</td>
<td>Melanoma²</td>
<td>2011</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>PD-1 mAb</td>
<td>Melanoma², non-small-cell lung cancer, Hodgkin lymphoma, advanced gastric cancer, microsatellite instability-high cancer, head and neck cancer and advanced urothelial bladder cancer</td>
<td>2014</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>PD-1 mAb</td>
<td>Melanoma², bladder cancer, classical Hodgkin lymphoma, colorectal cancer, hepatocellular cancer, non-small-cell lung cancer, kidney cancer, squamous cell carcinoma of the head and neck and urothelial cancer</td>
<td>2014</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>PD-L1 mAb</td>
<td>Urothelial cancer² and non-small-cell lung cancer</td>
<td>2016</td>
</tr>
<tr>
<td>Avelumab</td>
<td>PD-L1 mAb</td>
<td>Merkel cell carcinoma² and urothelial cancer</td>
<td>2017</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>PD-L1 mAb</td>
<td>Urothelial cancer² and non-small-cell lung cancer</td>
<td>2017</td>
</tr>
<tr>
<td><strong>Cytokines for lymphocyte promotion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron A</td>
<td>Recombinant IFNa2b</td>
<td>Hairy cell leukaemia², melanoma, follicular lymphoma, and AIDS-related Kaposi sarcoma</td>
<td>1986</td>
</tr>
<tr>
<td>Therapy</td>
<td>Type</td>
<td>Approved cancers</td>
<td>Year of first approval</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Roferon-A</td>
<td>Recombinant IFNα2a</td>
<td>Hairy cell leukaemia&lt;sup&gt;a&lt;/sup&gt;, chronic myelogenous leukaemia and AIDS-related Kaposi sarcoma</td>
<td>1986</td>
</tr>
<tr>
<td>Aldesleukin</td>
<td>Recombinant IL-2</td>
<td>Melanoma&lt;sup&gt;a&lt;/sup&gt; and kidney cancer</td>
<td>1992</td>
</tr>
<tr>
<td>Imiquimod</td>
<td>Stimulates TNF, IL-12 and IFNγ production&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Basal cell carcinoma</td>
<td>2004</td>
</tr>
</tbody>
</table>

**Engineered T cell therapies**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Approved cancers</th>
<th>Year of first approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tisagenlecleucel</td>
<td>CD19-specific CAR T cells</td>
<td>B cell acute lymphocytic leukaemia&lt;sup&gt;a&lt;/sup&gt; and non-Hodgkin lymphoma</td>
<td>2017</td>
</tr>
<tr>
<td>Axicabtagene ciloleucel</td>
<td>CD19-specific CAR T cells</td>
<td>Large B cell lymphoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2017</td>
</tr>
</tbody>
</table>

**Vaccines**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Approved cancers</th>
<th>Year of first approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sipuleucel-T</td>
<td>Autologous PBMCs activated with recombinant human PAP–GM-CSF</td>
<td>Prostate cancer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2010</td>
</tr>
<tr>
<td>Bacillus Calmette–Guérin</td>
<td>Strain of <em>Mycobacterium tuberculosis</em> variant <em>bovis</em></td>
<td>Bladder cancer</td>
<td>1990</td>
</tr>
</tbody>
</table>

**Oncolytic viruses**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Approved cancers</th>
<th>Year of first approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talimogene laherparepvec</td>
<td>Genetically modified HSV type 1 designed to replicate within tumours and produce GM-CSF</td>
<td>Melanoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2015</td>
</tr>
</tbody>
</table>

**Bispecific antibodies**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Approved cancers</th>
<th>Year of first approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blinatumomab</td>
<td>CD19 and CD3 bispecific antibody</td>
<td>B cell acute lymphocytic leukaemia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2014</td>
</tr>
</tbody>
</table>
Table 1: US Food and Drug Administration (FDA)-approved caner immunotherapies. CAR, chimeric antigen receptor; CTLA4, cytotoxic T lymphocyte antigen 4; GM-CSF, granulocyte–macrophage colony-stimulating factor; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; PAP, prostatic acid phosphatase; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death 1; PD-L1, PD-1 ligand 1. aFirst indication to be approved. bIncreases production of cytokines when topically applied. Figure adapted from Ref [8].

Drug delivery can be broadly categorized into local delivery and systemic delivery (Figure 1). In local drug delivery, the delivery system releases drugs or elicits a response in the immediate proximity of the material. The most used biomaterials in local drug delivery are hydrogels, scaffolds, and microparticles. Hydrogels and scaffolds provide a three-dimensional environment for immune cell recruitment and dendritic cell/antigen presenting cell (APC) programming using cancer antigens, as well as, spatiotemporal control over the release of immunomodulatory drugs at local sites. Microparticles provides an injectable platform for local immunotherapy with a targeted delivery system that focuses drug release at the local site and controls the release of the drug. On the other hand, drugs delivered systemically distribute throughout the body via the circulatory system. Systemic drug delivery biomaterials rely on an accumulation of the biomaterial at the tumor site, metastatic sites, and/or lymph nodes. These delivery devices typically include nanoparticles and drug conjugates. Nanoparticles are widely used in systemic drug delivery to deliver cancer vaccines and provide targeted immunomodulatory drug release to key immune cells. Nanoparticles help protect the agents and provides a customizable platform to suit numerous applications. Drug conjugation provides a simple approach to target delivery of immunotherapy drugs as well as modify the drug’s pharmacokinetics by
prolonging circulation and reducing clearance by the mononuclear phagocyte system (MPS).

To overcome the shortcomings of immunotherapy, immunomodulatory biomaterials can be developed as immunotherapy delivery systems to improve drug safety and efficacy\textsuperscript{[7-9]}. The application and design considerations of immunomodulatory biomaterials will be discussed to show the validity of these materials to improve immunotherapy treatment. Here, we review the various immunomodulatory biomaterials that can be applied in cancer immunotherapy – for both local and systemic delivery applications.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{local_systemic_delivery.png}
\caption{Figure 1: Representative figure of local and systemic drug delivery.}
\end{figure}
Local Immunomodulatory Biomaterials

When immunotherapy drugs are administered systemically, the patient can experience systemic toxicity and other off-target side effects. Therefore, researchers started to modify the immunotherapy drugs or package them in nanoparticles to increase their safety for systemic administration; however, these treatments often lack sufficient accumulation of the payload in tumors needed to elicit an antitumor immune response. To overcome these issues, researchers are investigating a more local approach to immunomodulation. To accomplish this, macroscale drug delivery devices and biomaterials have been developed as promising therapeutic avenues. Taking a local approach in immunomodulation of the tumor microenvironment (TME) allows for a focused administration of the cancer treatment that directly affects the tumor and immune cells that infiltrate the tumor. Current local immunomodulation biomaterials offer numerous advantages over systemic immunomodulation \[10-13\]. First, local immunomodulatory biomaterials only need low doses of immunomodulatory drugs/agents which circumvents the issue of systemic toxicity and other side effects, such as vascular leak syndrome and cytokine release syndrome. Local administration lowers the dose of drugs necessary due to the proximity to the treatment site and often incorporate a targeting mechanism. These targeting mechanisms can promote immune cell recruitment or target circulating immune cells (e.g. antibodies conjugated to the payloads within the local immunomodulatory biomaterials). In addition to the low dose requirement, local biomaterials spatiotemporally control drug release to optimize the immune response. Local
immunomodulatory biomaterials can be customized with a variety of chemical, mechanical, and physical properties that optimize the drug release profile. Of note, important properties that control drug release from local biomaterials include the rate of polymer degradation, diffusion mechanism, and affinity between the biomaterial and the drug \[^{12}\].

Many of the local biomaterials being developed can be classified as either hydrogels, scaffolds, or microparticles. Hydrogels and scaffolds provide a 3-dimensional (3D) environment that can be loaded with stimulatory factors to aid in the recruitment of APCs, such as DCs. After the host’s immune cells infiltrate the mesh network of the hydrogel or scaffold, immunomodulatory drugs such as cancer vaccines, anticancer antigens, or adjuvants can be presented to the immune cells to prime an antitumor immune response that hinders the growth or proliferation of cancer cells. Microparticles offer a different approach to administer cancer vaccines or immunotherapy drugs that can potentially target specific immune cells that are frequently found at the tumor site or target cancerous cells themselves to deliver the payload. Local immunomodulatory biomaterials have made significant strides in cancer treatment by changing the immunogenicity of the tumor and by equipping the immune system with the materials for a targeted and sustained antitumor response.

**Hydrogels**

Hydrogels are injectable biomaterials that can be made from various polymers, including natural polymers, synthetic polymers, or a hybrid composite of the two, that cross
links to form a 3D network. In comparison to the other local immunomodulatory biomaterials, hydrogels have high biocompatibility, biodegradability, and customizability. Another quality of note is the hydrophilic properties of hydrogels and their large swelling ratio that creates a conducive environment that aids in immune cell maturation and proliferation. Hydrogels can be used to hold numerous types of immunomodulatory agents depending on the properties of the polymers and the porosity of the hydrogel. Hydrogels can also be made responsive to environmental stimuli. Depending on the stimulus, it can cause the sol-gel transition of the hydrogel or could impact the drug release mechanism. The unique characteristics of hydrogels make them promising materials for drug delivery, sparking interest in various research areas, including tissue engineering \[20\] and cancer immunotherapy \[13,15-16\].

**Hydrogels: Design Considerations for Effective Drug Delivery**

In designing effective hydrogels for local immunomodulation, one must consider multiple parameters that will affect the drug release profile, biocompatibility, and the number of biological agents that can be loaded into the matrix. These parameters can include the polymer volume fraction in the hydrogel, the polymer type, the diffusion coefficient of the matrix, shear rate, and many more factors that can greatly impact the effectiveness of treatment \[13,15,17-18\]. Hydrogels can be used to immobilize numerous immunomodulatory agents; however, the size of the agents that can be stored within the polymer mesh of the hydrogel is controlled by the size of the meshwork and its porosity \[15\]. The porosity of hydrogels is determined by the distance between neighboring cross-links between polymers. As the number of cross-links (or the cross-linking density) increases within the hydrogel network, the size of the pores decreases, limiting the size of
biological agents that can be loaded into the hydrogel and impacting immune cell infiltration. Cross-linking density also dictates the shear rate, diffusion coefficient, and swelling volume of hydrogels. The shear rate of hydrogels affects the injectability of the biomaterial. Generally, the viscosity of the hydrogel solution increases as the weight percent of polymer is increased. The diffusional capabilities of hydrogel aids in the ability to provide a space for immune cell proliferation as well as a matrix to control the release of immunomodulatory agents. The swelling volume dictates the hydrogels ability to swell from the interactions between the hydrophilic polymers of the matrix and the aqueous ECM thus creating a conducive environment for immune cell infiltration and proliferation. A visual representation of the impact cross linking density has on the physical properties of the hydrogel can be found in Figure 2. Cross linking density is an important factor that impacts the deliverability of the hydrogel and subsequent drug release and immune cell infiltration.
Figure 2: Relationship between the cross-linking density and the physical properties of the hydrogel. Two visual representations of hydrogel structures with a low cross-linking density (left image) and a high cross-linking density (right image) are included along with a graphical representation of how the properties of the hydrogel change as cross-linking density increases. The properties include the shear modulus ($G$), equilibrium volumetric swelling ratio ($Q$), diffusivity ($D$), and mesh size ($\xi$). Figure adapted from Ref [15].

The chemical properties of hydrogels critically impact clinical application and effectiveness\(^{[18]}\). The charge and the hydrophilicity of the polymeric chains in the hydrogel affect hydrogel swelling from aqueous solutions such as water and biological interstitial fluid. Chemical properties of the hydrogel polymers influence compatibility between the biomaterial and the immunomodulatory agents. Chemical interactions, either repulsive or attractive, between the polymeric meshwork and the immunomodulatory agents impact the diffusional capabilities of the biomaterial. By modifying the chemical and physical properties of hydrogels, researchers can create a library of different hydrogels that are optimized for local immunomodulatory drug delivery.
Hydrogels: Synthetic and Natural Polymers

Hydrogels can be created using a variety of polymers categorized as natural-based, synthetic-based, or a combination of the two\textsuperscript{[13, 19-21,58]}. Natural polymers have intrinsic bioreactive and biocompatible properties that closely mimics the ECM. Natural polymers have a high degree of biodegradability and degrade into natural byproducts that are easily cleared by the body. Synthetic polymers typically are nonimmunogenic and often do not interact with the cellular environment. To encourage biological interactions, synthetic polymers can be conjugated to biological ligands and proteins recognizable by host cells. Some synthetic polymers pose a risk of toxicity\textsuperscript{[76]} because of issues of biocompatibility or with its degradation products, but many synthetic polymers used in hydrogel development are FDA-approved. These features of natural and synthetic polymers should be taken into consideration when developing an immunomodulatory hydrogel.

The choice to use a natural polymer or a synthetic polymer is based on a variety of physicochemical, mechanical, and biological parameters and guided by the intended use of the biomaterial. For instance, hydrogels made from collagen or gelatin are used widely in tissue engineering because of their prominent roles in the native extracellular matrix (ECM)\textsuperscript{[20]}. Collagen and gelatin have natural chemical properties that make it highly bioreactive and easily transition to a gel; however, these polymers generally form hydrogels that are structurally weaker than synthetic alternatives. To overcome limitations of hydrogels composed of purely natural or synthetic polymers, hybrid polymers are being studied to combine the best features of natural and synthetic polymers. In one study, researchers developed a hybrid hydrogel composed of levodopa, a stabilizing agent to slow biodegradation, and poly(ε-caprolactone-\textit{co}-lactide) ester-functionalized hyaluronic acid...
Hyaluronic acid (HA) is a natural polysaccharide and component of ECM in connective tissue that impacts biological processes such as cell migration and proliferation. Alone, hyaluronic acid hydrogels have high batch-to-batch variability, rapidly biodegrade (detrimental depending on the application), and are often contaminated. By combining HA and PCLA, the researchers were able to create a hydrogel that is capable of transitioning to a gel state when the solution reaches body temperature and retain the desired properties of HA, mainly its high biocompatibility. These hydrogels were able to deliver granulocyte-macrophage colony-stimulating factor (GM-CSF) to enhance immune cell recruitment to the hydrogel and a nanopolyplex-based DNA vaccine. To test the prophylactic capabilities of the hydrogel formulation, mice were first immunized with the hydrogel containing GM-CSF and the vaccine followed by an injection of B16/OVA melanoma cells a week later. As seen in Figure 3, mice immunized with the hydrogel containing both the recruiting cytokine and the vaccine had a significantly lower tumor index compared to free administration of the cytokine and vaccine (P < 0.001). Compared to hydrogels composed of only PCLA, using hybrid hydrogels composed of HA and PCLA significantly lowered the tumor index in the prophylactic study (P < 0.05). Using the hybrid hydrogels loaded with GM-CSF and the nanopolyplex-based vaccine elicited a strong antitumor response that provided continuous protection in some mice after 4 weeks from the melanoma inoculation. Hydrogels composed of only natural or synthetic polymers show promise as local immunomodulatory biomaterials; however, hybrid hydrogels offer a promising avenue to create novel delivery systems combining positive properties of each material class.
Figure 3: Prophylactic capability of nanoplex DNA vaccine against murine B16/OVA lung melanoma. C57BL/6 mice were inoculated with melanoma cells 1 week after receiving hydrogel-based cancer vaccine followed by a booster vaccine on day 14. Mice lungs were collected after 4 weeks and foci were quantified under dissecting microscope. Treatment groups from left to right are negative control, OVA-loaded HA-PCLA, (OVA + GM-CSF)-loaded HA-PCLA, free polyplex, free polyplex + GM-CSF, polyplex-loaded PCLA, (polyplex + GM-CSF)-loaded PCLA, polyplex-loaded HA-PCLA, (polyplex + GM-CSF)-loaded HA-PCLA. The graph above indicates the tumor index calculated as the average of (lung weights x grade) for each group. The error bars in the graph indicates mean ± SD (n = 4). Data were analyzed using Student’s t-test (*P < 0.05, **P < 0.01, ***P < 0.001). Figure adapted from Ref [22].

Hydrogels: Stimuli-Responsive “Smart” Hydrogels

The customizability of hydrogels allows for hydrogels to be designed to respond to various environmental stimuli, including pH, temperature, oxidative stress, and enzymatic activity, among other cues [58]. For example, a temperature stimulus can be utilized to trigger the sol-gel transition after injection of the hydrogel solution. Once the solution reaches body temperature, the solution will become more viscous and swell until it reaches
a gel state. This phase change is due to a destabilizing effect on the hydrophobic interactions between the polymers and hydrophilic interactions with the surrounding extracellular fluid. The thermoresponsive property of hydrogels is affected by the concentration of thermoresponsive polymers. In one study, researchers developed a thermoresponsive triblock copolymer hydrogel composed of poly(γ-ethyl-L-glutamate) (PELG) and poly (ethylene glycol) (PEG) arranged as PELG-b-PEG-b-PELG [23]. The required temperature to start the sol-to-gel phase transition decreased as the polymer concentration increased from 3 wt% to 6 wt% of the hydrogel solution. Thus, changes as simple as the polymer concentration in hydrogel solutions cause changes in the gelling process and must be taken into consideration when designing an injectable system. Carefully modifying the sol-gel transition allows for the hydrogel to be injected at the target site and form the biomimicking matrix to recruit immune cells or deliver immunomodulatory agents.

The pH of the microenvironment can cause certain hydrogels to switch chemical, mechanical, and physical properties. These pH responsive hydrogels respond differently to pH levels depending on the functional groups associated with the polymer [13,18]. Polymers with acidic groups cause hydrogels to swell as pH increases due to the deprotonation of acidic R groups. On the other hand, polymers with basic R groups cause hydrogels to swell when the pH decreases. Stimuli-responsive hydrogels offer “smart” systems capable of responding to their microenvironment and tightly regulating material response based on biological processes occurring within the microenvironment.
Scaffolds

Scaffolds are 3D polymeric networks with applications in host cell recruitment and spatiotemporal drug release. Scaffolds and hydrogels have similar functional properties that allow for researchers to interchange between the two platforms. Scaffolds can be used in a variety of ways to enhance local immunomodulation. Polymeric scaffolds loaded with recruitment factors, such as GM-CSF, can promote immune cell recruitment into the scaffold matrix where the immune cells can be exposed to cancer vaccines, antitumor antigens, or adjuvants to aid in immune cell maturation. Afterward, those newly programmed immune cells can leave the scaffold to aid in an antitumor immune response. For example, in an intriguing study Ali et al. developed a macroporous poly-lactide-co-glycolide (PLG) scaffold loaded with GM-CSF, danger signals (unmethylated cytosine-phosphate-guanine oligonucleotide, or CpG-ODN), and tumor antigens to recruit and reprogram DCs to elicit an antitumor response \(^{[24]}\) (Figure 4). A dendritic cell-activating scaffold, based on the technology developed by Ali et al., that contains melanoma cell lysates is currently in phase I clinical testing. Scaffolds have also been used to act as a delivery system for T-cells programmed \textit{in vitro} such as CAR-T cell therapies \(^{[25]}\). These scaffolds, loaded with CAR-T cells and STING agonists, improve T cell infiltration into solid tumors and improve the elimination of malignant tumor cells. Scaffolds are generally fabricated \textit{ex vivo} and then must be implanted at the target site; however, there are some formulations of scaffolds that are injectable, specifically mesoporous silica rods (MSRs) \(^{[26,51]}\).
**Figure 4:** Process schematic of Ali et. al. infection-mimicking scaffold design. Stage 1: Recruit naïve dendritic cells and APCs using released GM-CSF. Stage 2: The recruited dendritic cells/APCs reside in the matrix of the scaffold to be programmed using preloaded cancer antigens. Stage 3: The newly programmed dendritic cells/APCs leave the scaffold to activate T-cells and initiate an anticancer immune response.

**Scaffolds: Injectable vs. Surgical**

Surgery has always been a prominent part of the treatment course for oncology patients that has curative results, but surgical procedures are not without risk. Implantable scaffolds are often placed either at the tumor resection site to lower the chances of relapse or placed subcutaneously near a lymph node to recruit and reprogram immune cells. Many of the implantable scaffolds in development are composed of PLG due to its long-standing
FDA approval, record of biocompatibility, and material tunability. In one study of note on implantable scaffolds, the 3D printed PLGA scaffold developed by Yang et al. acted as a drug delivery device for combination chemotherapy using 5-fluorouracil and NVP-BEZ235, known as PFN scaffolds ([48]). Like immunotherapy drugs, chemotherapy drugs lead to systemic toxicity at high doses. By incorporating chemotherapy locally via the polymeric scaffold, researchers created a local drug delivery system to influence the TME while diminishing systemic toxicity. In a therapeutic efficacy study conducted over 4 weeks, the average tumor volume of mice that received the PFN scaffold was 600 mm$^3$ compared to an average tumor volume of 1000 mm$^3$ in mice that received an intraperitoneal injection of both chemotherapeutic drugs at equal concentrations every 3 days. This combinational therapy delivered by the PLGA scaffold effectively slowed tumor growth in a murine MDA-MB-231 orthotopic breast cancer model.

To avoid the limitations of surgically-implanted materials (e.g. inaccessible tumor sites, infection), injectable scaffolds are being developed and studied to create local immunogenic treatments on par with implantable scaffolds. Injectable scaffolds offer many advantages over implantable scaffolds, particularly the ability to access hard-to-reach tumors that implantable scaffolds cannot reach. Some tumors are inoperable, so implanting a scaffold to aid in an immunotherapy cancer treatment would be hindered. However, injectable scaffolds could be placed close to inoperable tumor sites to enhance cancer treatment. Injectable scaffolds have been developed using materials including alginate ([49], gelatin ([50], and mesoporous silica rods (MSRs) ([26,51], among other materials. Injectable scaffold materials are injected as a solution before rapidly assembling into a 3D matrix in vivo that can recruit and activate immune cells or act as immunomodulatory drug
reservoirs. For example, injectable MSRs with a high aspect ratio can spontaneously form a macroporous 3D scaffold to allow for immune cell recruitment and modulation. In a study done by Kim and colleagues, injectable high-aspect-ratio MSRs were developed to assemble *in vivo* and recruit host immune cells by releasing GM-CSF into the surrounding tissue [26]. After recruitment, the immune cells, primarily DCs, could be matured using CpG-ODN and protein antigens. Kim and colleagues assessed the ability of the MSR system to induce antigen-specific adaptive immune responses. The MSR loaded with OVA, GM-CSF, and CpG-ODN produced strong titers for sera anti-OVA IgG2a and IgG1 which corresponds to strong Th1 and Th2 responses, respectively. The injectable MSR system the researchers developed performed better than the control bolus model containing only the vaccine or OVA and showed improved humoral and adaptive immune responses. To further solidify the effectiveness of the purposed MSR vaccine system, Kim and colleagues conducted a study to investigate the ability of the delivery system to produce an antitumor immune response (*Figure 5*). Mice were vaccinated with MSR vaccines then later inoculated with EG7.OVA lymphoma cells. Mice vaccinated using MSR scaffolds loaded with OVA, GM-CSF, and CpG-ODN had tumors with significantly smaller volumes compared to bolus injection of the vaccine (P < 0.05), MSR scaffolds loaded with OVA only (P < 0.01), and blank MSR scaffolds (P < 0.001).
Figure 5: Prophylactic cancer vaccine study using injectable, spontaneously forming MSR scaffolds. C57BL/6J mice received MSR vaccine and were challenged 10 days post-vaccination with EG7.OVA lymphoma cells in the back of the neck. EG7.OVA tumor volume (A) and survival rate (B) were monitored after tumor inoculation. In graph A, tumor volumes between treatment groups were compared on days 21, 23, and 25. Error bars represent mean ± s.e.m. (n = 10). Data analyzed using Student’s t-test (*P < 0.05, **P < 0.01, ***P < 0.001). Figure adapted from Ref [26].

Scaffolds: Design Parameters

Like hydrogels, modifying different design parameters of scaffolds can change the physical properties of the matrix, impact diffusivity of immunomodulatory factors to surrounding tissue, and bioreactivity. The physical properties of the scaffold are principally impacted by the choice of polymer and the fabrication process. Polymers have a specific
set of physical properties that are important in determining the structural stability of the scaffold when placed within the human body. The scaffold must be able to retain its form and avoid a premature collapse of the 3D matrix. In addition to considering the mechanical properties of the scaffold, designers also must consider the diffusive properties of the matrix. Like hydrogels, diffusion is an important factor for scaffolds in aiding immune cell recruitment and survival as well as controlled drug release. The diffusion coefficient, as well as the drug loading capability, of the scaffold is dependent on the porosity of the matrix. The pore size of scaffolds depends highly on the fabrication method used. For instance, one method to create a 3D matrix in the scaffold is by sparging air or carbon dioxide as the scaffold sets. This creates bubbles/pores in the scaffold to allow for diffusion and drug loading \cite{52}. Other variations of this fabrication exist including gas generation from the crosslinking process. One of the simplest and oldest methods of fabrication is particulate leaching, or salt leaching \cite{52}. In this method, salt or other porogens are poured into a mold followed by the polymer solution. Once the solvent evaporates, the salt is leached away with water leaving pores in the scaffold. A more controllable fabrication method is the use of 3D printing to create a crosslinking lattice. In the study done by Yang et. al., the PFN scaffold was fabricated using an E-jet 3D printer \cite{48}. The porosity of the scaffolds impacts the drug release profile of the delivery device. Researchers created various scaffolds with different degrees of porosity by using different aperture sizes. Their studied showed that as aperture size increased the porosity of the scaffold increased.

Scaffolds can be engineered by many approaches to improve immune cell recruitment and spatiotemporal drug release. One such route is to modify scaffold porosity. Injectable high-aspect-ratio MSRs nonspecifically assemble to form 3D structures
containing interparticle spaces, or pores. For example, researchers used MSRs with a hexagonal mesoporous structure and upon injection with a pore-directing agent, Pluronic® P-123, scaffolds with a 3D microenvironment were produced [26]. These researchers also tested the effect of mesopores and macropores in immune cell recruitment by looking at cell recruitment in 2 materials: a pore-filled silica microrod with similar qualities to MSRs but lacking the mesoporosity of pristine MSRs and a pressed MSR that preserved the mesopores but lacked macropores. Equal masses of the pore-filled MSRs, pressed MSRs, and untampered MSRs were injected into mice and the number of host cells recruited into the scaffold was analyzed on day 3. The pristine MSRs recruited approximately $2.4 \times 10^6$ cells which was significantly higher than the pore-filled and pressed MSRs ($P < 0.05$). When comparing pore-filled MSRs to pressed MSRs, pore-filled MSRs recruited approximately $0.4 \times 10^6$ more cells than pressed MSRs. This data indicates that interparticle macropores were vital in cell recruitment into the scaffold.

Another way to impact the efficacy of scaffolds is through surface modification. Some synthetic polymers used as the basis of the scaffold have poor bioreactivity which can hinder cell recruitment and activation; however, this can be changed through surface modification. Aileen Li et. al. conducted a study analyzing the effect of surface modification of MSR scaffolds on immune cell recruitment and programming [27]. They modified the scaffold with PEG, PEG-RGD (integrin-binding ligand Arg-Gly-Asp), and PEG-RDG (Arg-Asp-Gly) groups. Mice received subcutaneous injections of either unmodified MSRs or one of the surface-modified MSRs. On day 5, the scaffolds were explanted and analyzed. The total cell content of PEG modified MSRs were significantly higher than the cell content of unmodified MSRs ($P < 0.05$). The total number of cells
infiltrating PEG-modified MSRs was 10 times greater than unmodified MSRs and 4 times greater than PEG-RGD MSRs or PEG-RDG MSRs. Next, the type of immune cells that infiltrated that scaffold was analyzed. PEG MSRs contained a significantly lower mean percentage of DCs (< 1%) compared to unmodified MSRs (~4%), but the total number of DCs was not significantly different between the MSR scaffolds. A majority of the infiltrating immune cells were myeloid cells/neutrophils. PEG modified MSR scaffolds had a mean percentage of approximately 75%, significantly higher than the mean percentage of myeloid cells/neutrophils that infiltrated the unmodified MSRs (~63%), PEG-RGD-modified MSRs (~53%), and PEG-RDG-modified MSRs (~53%) (P < 0.05). MSRs modified using PEG displayed increased inflammatory responses which in turn increased immune cell recruitment. On the other hand, scaffolds modified with PEG-RGD and PEG-RDG showed decrease immune responses likely due to a hindrance in interactions between PEG and components of the ECM.

**Microparticles**

Microparticles are substantially smaller than hydrogels and scaffolds and are normally used to encapsulate various immunomodulatory agents including immunotherapy drugs and cancer vaccines. Microparticles typically range from ~1 μm to 50 μm in diameter. The large size of microparticles impacts particle diffusion and interaction with host cells. For instance, microparticles are taken up by immune cells, specifically APCs, via phagocytosis while smaller delivery systems such as nanoparticles can be taken up via endocytosis and micropinocytosis. These particles can be loaded with cancer antigens and/or immunomodulatory drugs and tagged with antibodies to target immune cells. When
used as a cancer vaccine delivery system, microparticles protect the antigen from degradation normally associated with a bolus injection and are a good platform for synergistic combination therapies to enhance an antitumor response. Microparticles can serve both as local and systemic immunomodulatory biomaterials, but systemic applications are limited due to the large relative size of microparticles, i.e. they are too large to circulate. As a local immunomodulatory biomaterial, microparticles reside in the target/injection site to deliver therapeutics in a controlled, often sustained manner using a variety of drug release mechanisms (Figure 6). In this context, microparticles can be used alone or in conjunction with hydrogels and scaffolds. For instance, Davoodi et. al. formulated a treatment system that utilized a core-shell polymeric microparticle encapsulating cisplatin and paclitaxel embedded in an injectable hydrogel to create a novel localized delivery system to treat triple negative breast cancer [54]. Microparticles provide a modifiable platform to optimize local drug delivery of immunotherapeutic.
Figure 6: Various release mechanisms of microparticle systems. Active pharmaceutical ingredient (API). Figure adapted from Ref [79].

_Microparticles: Types of Microparticles and Their Applications_

Microparticles can be fabricated using a variety of methods, including organism-derived, natural, and synthetic microparticles [12]. Organism-derived microparticles are released by cells, collected, and purified into the final material. Exosomes – extracellular...
vesicles with cellular origin – are an example of organism-derived microparticles. Tumors release exosomes, containing tumor-specific antigens, when exposed to an external stimulus that can be used as a vaccine. For example, Zhao et. al. created tumor microparticles (T-MPs) by exposing B16F10 tumor cells to UV radiation \cite{28}. After release, the exosomes were loaded with nano-sized Fe$_3$O$_4$ and CpG-loaded liposomes were attached to the surface of exosomes. To assess the capabilities of the Fe$_3$O$_4$/T-MPs-CpG/Lipo vaccine to produce an antitumor response, the researchers conducted a prophylactic study using the B16F10 melanoma tumor model (Figure 7). Mice were vaccinated on days 1, 2, and 7 then received an inoculation of B16F10 cells on day 8. 85.7% of the mice vaccinated with the Fe$_3$O$_4$/T-MPs-CpG/Lipo vaccine formulation remained tumor-free by the end of the study.
**Figure 7:** Murine B16F10 melanoma prophylactic study using Fe$_3$O$_4$/T-MPs-CpG/Lipo vaccine. C57BL/6 mice were vaccinated on days 1, 2, and 7 followed by melanoma cell inoculation on day 8 (A). Average tumor growth curves (B) and the survival rate (C) were calculated during the experimental time. Error bars represent mean ± SD (n = 7). Data in graph B was analyzed using two-way ANOVA with the Bonferroni’s multiple comparison post-test and data in graph C was analyzed using the log-rank test (*P < 0.05, **P < 0.01, ***P < 0.001). Figure adapted from Ref [28].

On day 20, tumor tissue was extracted and analyzed to assess the immunogenicity of the tumor. To determine the switch from a “cold” tumor to a “hot” tumor, researchers looked at a phenotypic switch in macrophages from pro-cancer M2 macrophages to anti-cancer
M1 macrophages. Vaccination with the Fe$_3$O$_4$/T-MPs-CpG/Lipo formulation enhanced M1 macrophage surface marker expression compared to an empty control and the T-MPs-CpG/Lipo vaccine. The researchers also analyzed cell suspensions from the lymph nodes and spleen to further demonstrate the ability of the Fe$_3$O$_4$/T-MPs-CpG/Lipo vaccine to switch macrophages to an M1 phenotype. Based on a one-way ANOVA with Bonferroni’s post-test, the percent of M1 macrophages was significantly higher in mice vaccinated with the Fe$_3$O$_4$/T-MPs-CpG/Lipo vaccine compared to an empty control (P < 0.001) and the T-MPs-CpG/Lipo vaccine (P < 0.01). By creating this newly modified tumor exosome/microparticle, the researchers were able to elicit a strong immune response that increased the immunogenicity of the TME and inhibited tumor growth.

Natural polymer-based microparticles, such as alginate and chitosan, are biocompatible and readily tunable to allow for variable drug release mechanisms. In an early study by Lin-Shu Liu et. al., porous microspheres formed from alginate and chitosan were loaded with IL-2 to study the drug release profile and bioactivity of the released cytokine overtime [29]. The researchers conducted a protein release study with 3 formulations of alginate microspheres using CaCl$_2$, chitosan, and polylysine. These alginate microspheres were loaded with different amounts of proteins including albumin, bovine-fluorescein isothiocyanate (FITC-BSA), and IL-2. With a FITC-BSA protein loading of 10 wt%, alginate/chitosan microspheres released 45% of the payload within 6 hrs compared to nearly 100% release by alginate/CaCl$_2$ microspheres and 75% release by alginate/polylysine. Alginate/chitosan microspheres steadily released FITC-BSA over 4 days compared to the rapid release of the protein from the other microspheres within 2 days. Based on this preliminary data, alginate/chitosan microspheres showed promising
drug release profiles, so the researchers assessed release of IL-2 from these materials. Over a period of 5 days, alginate/chitosan microspheres steadily released 100% of the loaded IL-2. After investigating the protein release profile of the alginate microspheres, the researchers assessed the ability of the alginate/chitosan microspheres to activate cytotoxic T lymphocytes. In this experiment, either microspheres loaded with IL-2 or free IL-2 were added to a cell culture of human lung squamous carcinoma SQ-5 cells and peripheral blood mononuclear monocytes. Activated lymphocytes were isolated from the culture at 1 week post treatment and 1 month post treatment to assess T cell activation over both short-term and long-term culture periods. After 1 week, both the microsphere delivery system and free administration of IL-2 yielded 4.5 * 10^7 activated T cells. After the 1-month culture period, the microsphere system yielded 80 * 10^7 activated T cells compared to 47 * 10^7 activated T cells by free IL-2. Alginate/chitosan microspheres steadily released IL-2 over time and effectively generated more activated T cells compared to free IL-2 during longer culture periods.

Synthetic polymer-based microparticles are highly customizable and readily available for use. In a study by Rahimian et. al., poly (lactic-co-hydroxymethyl-glycolic acid) (pLHMGGA) microparticles were loaded with either antiCD40 or antiCTLA-4 immunomodulatory antibodies. During an in vitro antibody release study, the microparticle initially released about 20% of the antibody payloads followed by a sustained release of antiCD40 or antiCTLA-4 reaching 80% drug release by day 30. The researchers also investigated the therapeutic efficacy of the microparticles, compared to incomplete Freund’s adjuvant (IFA), by treating mice inoculated with MC-38 cells, a colon carcinoma tumor model, when the tumor became palpable (Figure 8). 50% of the mice survived that
received antiCD40 antibody treatment from either IFA or from the microparticles compared to 10 \% survival in untreated mice. A similar trend occurs in mice treated with antiCTLA-4 antibody treatment. 40\% of the mice treated using microparticles survived and 30 \% of the mice treated with using IFA survived. Compared to untreated mice, significantly more mice survived after treatment with antiCD40 (P < 0.001) and antiCTLA-4 (P < 0.01). These microparticles increased the survival rate of mice inoculated with MC-38 tumors that was comparable to common IFA formulations. In sum, polymer chemistry and source play integral roles in determining the application of microparticles in cancer immunotherapy.

**Figure 8:** Therapeutic efficacy of antiCD40 and antiCTLA-4 microparticle formulation against MC-38 tumor cells. Kaplan-Meier plots presenting the survival proportions of tumor bearing mice treated with different microparticle formulations containing antiCD40 (A) and antiCTLA-4 (B). Data from two experiments (n = 14-18). Data analyzed using log-rank test (*P < 0.05, **P < 0.01, ***P < 0.001). Figure adapted from Ref [30].
**Microparticles: Design Considerations**

The application of the microparticle greatly influences which design parameters to consider during fabrication. After injection, microparticles will either interact with immune cells, specifically phagocytes and APCs, or act as an immunomodulatory agent reservoir, providing controlled and sustained drug release. Microparticles that act as cancer vaccines or tumor antigen reservoirs should be taken in by APCs through phagocytosis. Once inside the APC, the microparticle is broken down in an endosome and loads freed antigen to Major histocompatibility complex (MHC) I and II to start the maturation process for an antitumor response. In a study conducted by Foged et. al., the role of particle size and surface charge in microparticle uptake by human DCs was investigated \[^{31}\]. The researchers used polystyrene spheres with diameters of 0.1, 0.5, 1.0, and 4.5 μm to model microparticles. To assess the interaction between microparticles and dendritic cells (DCs), microparticles of various diameters were incubated for 24 hours with DCs and analyzed by flow cytometry to quantify the amount of double positive cells (indicative of microparticles bound to the surface of the DCs). From the flow cytometry analysis, less than 5% of DCs bonded with microparticles with a diameter of 4.5 μm, 10% of the DCs interacted with 1.0 μm microparticles, 30% of the DCs bonded with 0.5 μm microparticles, and 60% associated with 0.1 μm microparticles. As particle size decreased, the number of polystyrene spheres bound to DCs, thus potentially endocytosed, increased. In the case of surface charge, different polyaminoacids/proteins were conjugated to the surface of polystyrene spheres with a diameter of 1 and 0.1 μm. The negatively charged particles interacted less with DCs compared to the particles with a positive surface charge. Based on their results, the researchers saw that surface charge played a bigger role in DC interaction for large particles.
suggesting that modifying larger particles to have a positive charge could enhance DC uptake. On the other hand, microparticles that provide sustained release of immunomodulatory agents should avoid being internalized by phagocytes. For this reason, many physicochemical and mechanical properties can be considered to discourage microparticle clearance by phagocytes. A review paper to note by Moon et. al. provides an in-depth review of the impact of particle shape and mechanical properties on phagocyte interactions [32]. Microparticles are a valuable local immunomodulatory biomaterial with broad applications due to their simplicity and elegance.
Systemic Immunomodulatory Biomaterials

Systemic administration of immunomodulatory drugs is a promising approach for the treatment of metastatic cancers that has spread to distant sites throughout the body as well as the treatment of primary tumors. Accordingly, the U.S. Food and Drug Administration has approved many immunotherapy drugs to treat metastatic cancer, *e.g.* interferons, interleukins, toll-like receptor agonists, and immune checkpoint inhibitors [3,5,55-56]. Even though these immunotherapies have gained FDA approval, systemic administration of the drugs have multiple drawbacks [55-56]. The dose of immunomodulatory drugs given systemically is limited by concerns about toxicity [57]. Additionally, large portions of the systemically administered drugs fail to reach the target site, instead biodistributing to other organs, limiting on-target efficacy and increasing off-target toxicity [58-59]. Immunomodulatory drugs are rapidly excreted, degraded as they circulate, and accumulate at off-target sites [59]. To address the poor natural pharmacokinetics and biodistribution of many immunotherapeutic drugs, numerous immunomodulatory biomaterials have been developed for systemic delivery. Here, we focus our attention on two major classes of systemic delivery materials: nanoparticles and drug conjugates.

Nanoparticle technology has been widely studied for the delivery of immunomodulatory drugs. Nanoparticles contain many customizable features that can be modified to: target specific immune cells or cancer cells, degrade at a pre-programmed or
adjustable/responsive rate, protect the immunomodulatory drug in circulation, and improve biodistribution to lymph nodes and/or tumors. Nanoparticles are an especially promising delivery system because 1) they can leverage the enhanced permeability and retention (EPR) effect to target tumors \([60-61]\) and 2) recent reports show efficient accumulation of nanoparticles in tumor-associated leukocytes \([62-63]\).

Drug conjugation modifies the immunomodulatory drug itself to improve pharmacokinetics and biodistribution. Immunomodulatory drugs can be conjugated to monoclonal antibodies to target the drug to key receptors that are predominantly expressed on leukocytes or cancerous cells. This targeting strategy reduces off-target accumulation and can thus reduce systemic toxicity that results from off-target effects \([59]\). Immunomodulatory drugs can also be conjugated to synthetic polymers that serve to protect the drug from the harsh environment of the circulatory system as well as enhance the pharmacokinetics of the drug (\textit{i.e.} increase the circulation half-life) \([59]\). By protecting immunomodulatory drugs using nanoparticles or conjugation methods, researchers can improve the efficacy of the drug, reduce dose-limiting toxicities, and create a delivery platform that is tunable to a variety of applications.

**Nanoparticles**

Due to the systemic administration of nanoparticles, they are able to interact with a wide range of targets and elicit multifaceted immune responses \([9,12,64]\). For instance, nanoparticles can be leveraged to guide biodistribution to regional lymph nodes \([65]\) and target immunotherapeutics to a primary tumor \([36,61]\). A central goal in cancer
immunotherapy is to activate T-cells to seek and eliminate both local and metastatic cancerous cells. To achieve this goal, nanoparticles are used to deliver cancer vaccines, antigens, and adjuvants to APCs, deliver agonists to T-cells directly, imitate APCs to initiate T-cell proliferation, and deliver immune checkpoint inhibitors. In addition to T-cell activation, nanoparticles protect immunotherapy drugs from degradation in the blood and enhance treatment by prolonging circulation time by focusing the drug biodistribution to specific targets. Importantly, a wide range of nanoparticles have been developed that are either approved by the FDA or currently progressing through clinical trials. By continued improvement in our understanding of tumor immunobiology, advancements in materials chemistry and nanotechnology, and effective interdisciplinary collaboration between immunologists and biomedical engineers, the development of more FDA-approved products that can have a major impact on cancer immunotherapy are underway.

Immunotherapeutic drugs face a wide range of obstacles, such as cellular uptake and trafficking barriers, depending upon the type of drug (e.g. small molecule, antibody, nucleic acid) and intracellular destination (Figure 9). Upon encountering an appropriate APC, nanoparticles should trigger internalization into the targeted cell. Upon internalization, fate of the nanoparticles and accompanying immunotherapeutic drugs can be tuned to suit the intended application. For instance, immunotherapies for endosomal targets (e.g. TLR7/8 agonists and antigens for MHC-II) necessitate cell uptake via endocytosis but do not require endosomal escape, whereas immunotherapies for cytosolic targets (e.g. 5’pppRNA and mRNA vaccines) require both cell uptake and efficient endosomal escape. In a study done by Oberli et al., lipid nanoparticles were used to transfect APCs with an mRNA vaccine coding for two melanoma self-antigens: tyrosinase-

34
related protein 2 (Trp2) and glycoprotein 100 (gp100) with a point mutation. Vaccination with the lipid nanoparticles greatly increased the number of transfected APCs and increased CD8 T cell proliferation thus increasing the survival rate of mice with B16F10 tumors. By using a lipid nanoparticle, the researchers were able to effectively address the delivery challenges of using mRNA vaccines. The lipid nanoparticles employed by this group consisted of 5 components: an ionizable lipid, a phospholipid, cholesterol, PEG containing lipids, and an additive for mRNA vaccine delivery. Each of these components aid in the processes of biodistribution, cellular uptake, and endosomal escape. The ionizable lipid becomes positively charged at lower pH to aid in conjugation to mRNA and the positive charge aids in cellular uptake and endosomal escape. By incorporating phospholipids and cholesterol into the lipid nanoparticle, researchers help stabilize the particle and help with endosomal escape. By using PEG-lipid conjugates in the nanoparticle, the researchers discourage nanoparticle aggregation thus aiding in biodistribution.
Figure 9: Schematic of cell targeted delivery of immunotherapeutic agents using nanoparticles. Composed of three steps: i) the nanoparticle binds to specific receptors of the cell, ii) endocytic uptake of the nanoparticle through receptor-mediated endocytosis, and iii) immunotherapeutic agent release via endosomal escape. Figure adapted from Ref [39].

In another study done by Xu et. al., researchers used a nanoparticle composed of a calcium phosphate (CaP) core and an asymmetric lipid bilayer (lipid-calcium-phosphate (LCP) nanoparticle) to deliver a peptide vaccine of Trp2 to mouse models of melanoma (B16F10) [35]. The goal of the study was to elicit MHC I-restricted cytotoxic T-lymphocyte
responses to eradicate B16F10 tumors. In order for the peptide to be effective in creating an immune response, the nanoparticle has to deliver the vaccine to the cytosol of the APC. To achieve this goal, Xu and colleagues modified the surface of the nanoparticle with mannose to bind to mannose receptors of APCs and aid in cellular uptake. Additionally, LCP nanoparticles have two mechanisms of endosomal escape depending on the number of nanoparticles taken in by the APC \[66\]. If large quantities of LCP nanoparticles are endocytosed, endosomal escape occurs due to an increase in osmotic pressure in the acidic environment of the endosome that results in the dissolution of the CaP core. If smaller quantities of LCP nanoparticles are endocytosed, endosomal escape results from the formation of ion pairs between the cationic lipids in the asymmetric bilayer, dioleoylphosphatidylcholine (DOTAP), and negatively charged groups in the endosomal membrane. On the other hand, some nanoparticles do not have to be endocytosed by APCs to elicit an immune response. For example, researchers can conjugate MHC and antigen complexes to the surface of nanoparticles to create artificial APCs that can activate T cells \[67\]. By following similar design processes, nanoparticles can be synthesized that efficiently deliver immunotherapeutics to a variety of intracellular targets; endosomal, cytosolic, and otherwise.

*Nanoparticles: Design Considerations*

Nanoparticles can be fabricated using a variety of methods; however, there are key design parameters that must be considered in order to create an effective delivery system. The size of nanoparticles plays a key role in nanoparticle accumulation at tumor sites and clearance by phagocytes. Though the EPR effect is variable and mechanisms to better
understand nanoparticle accumulation in tumors are being investigated, tumors that contain leaky vasculature and impaired lymphatic drainage allow circulating nanoparticles to preferentially infiltrate the tumor and avoid clearance from the tumor site. One study of note was conducted by Perrault et. al. to systematically study how particle size (10-100 nm) influenced the pharmacokinetics of nanoparticles [37]. One of the experiments conducted by the researchers investigated the tumor accumulation of nanoparticles with varying sizes (20 nm, 40 nm, 60 nm, 80 nm, and 100 nm). Each size bracket showed different levels of accumulation in the tumor site; however, the researchers saw no clear trend between particle size and tumor accumulation. In the researchers’ model, the lack of correlation between particle size and tumor site accumulation should be reflected by a dependence on blood/circulation half-life over time. After doing a regression analysis, the researchers found that this phenomenon was only significant for particle sizes ranging from 40-100 nm (P < 0.02). This suggests that the size of smaller nanoparticles may affect tumor accumulation. To investigate this further, the researchers conducted another regression analysis that considered volumetric size of the nanoparticles (nm$^3$) and half-life. This analysis revealed a significant relationship in particle sizes ranging from 20 nm to 100 nm diameter in relation to half-life (P < 0.015). This regression analysis showed that the accumulation of nanoparticles around 20 nm depends on its size and half-life. On the other hand, particles ranging from 40-100 nm depends almost entirely on circulation half-life indicating a possible route to modify tumor accumulation by modifying pharmacokinetic parameters. Another important design factor brought to light by the study is the impact of size on the permeation of nanoparticles. Generally, as particle size increased, the area of permeation within tumors became smaller. In summation, these results show that
nanoparticle size profoundly impacts the amount of nanoparticle accumulation and homogeneity in the tumor site. Thus, researchers must determine the acceptable size range for nanoparticles to optimize accumulation and permeation for their particular application—though for most cases nanoparticles in smaller size ranges (~20-50 nm) appear most suitable.

Other key design considerations include the material of the particle, surface charge, and degradation mechanism \[38,39\]. For instance, nanoparticles composed of PEG are generally non-toxic and non-immunogenic, whereas poly(beta-amino esters) becomes immunogenic as they are degraded over time \[76\]. Surface charge is an important parameter with regards to cellular uptake and circulation time. Nanoparticles with a positively-charged surface have a higher rate of cellular uptake while neutral and slightly negatively-charged surfaces reduce cellular uptake. Due to the increased cellular uptake of positively-charged nanoparticles, in addition to rapid protein adsorption and aggregation, these particles generally have very short circulation times. For this reason, charged materials are often coated with materials like PEG to shield surface charge, increase biocompatibility, and prevent particle clearance by the MPS system. The degradation mechanism and drug release trigger can be modified to suit a wide range of applications. Some of these triggers include tumor hypoxia, low pH of endosomes and TME, tumor-specific enzymes, and oxidative stress. These triggers cause biodegradation of the nanoparticle material while also enabling a mechanism for drug release from the particles. For further analysis, please see the extensive review on linker chemistry design and nanoparticle drug release by Wong and Choi \[39\].
Nanoparticles: Types of nanoparticles and Their Applications

Nanoparticles are one of the most customizable biomaterials/delivery systems used in research. They can be created from a range of polymers and biological agents including synthetic polymers like PEG, lipids/lipid-like materials, natural polymers like hyaluronic acid, and inorganic metals like gold \[64\]. Similar to composite hydrogels and scaffolds, hybrid nanoparticles can be produced by combining different material classes into composites to leverage positive characteristics of each material. Researchers have engineered many types of nanoparticles including silica nanoparticles, dendrimers, carbon nanoparticles, ceramic nanoparticles, etc. \[68,69\]. The three types of nanoparticles discussed here are lipid-based, synthetic polymer-based, and natural polymer-based.

Lipid-based nanoparticles and liposomes are primarily composed of lipids and follow the design criteria of vesicles. The lipid-based nanoparticles can be composed of an ionizable lipid, phospholipids, cholesterol, PEG-lipids, and additives to aid in drug/vaccine delivery. Liposomes are primarily composed of either natural or synthetic lipids. They can have single or multiple lipid bilayers with an aqueous core to house both hydrophobic and hydrophilic immunomodulatory agents/drugs. A key advantage of lipid nanoparticles is the ease of conjugating ligands to the surface of the particle. The conjugation of ligands gives nanoparticles the ability to target key immune cells and encourage cellular uptake. In a study done by Zhang et. al., the surface of PEGylated liposomes were coated with IL-2 agonists and anti-CD137 ligands to stimulate the proliferation of cytotoxic T lymphocytes and natural killer (NK) cells as well as act as a co-stimulatory signal for T cell activation \[40\]. The researchers first assessed the effectiveness of a combinational treatment with anti-CD137 and IL-2-Fc, a fusion of the Fc domain to IL-2 to prolong circulation half-life,
against the B16F10 melanoma model. Mice with established B16F10 tumors received systemic injections of 20 μg of IL-2-Fc and 100 μg of anti-CD137 every 2 days for a total of 3 doses. The mice treated with anti-CD137/IL-2-Fc had smaller average tumor sizes compared to untreated mice; however, the mice experience severe systemic toxicity along with rapid weight loss that ultimately led to death by the third injection. Additionally, using a longer time interval between doses (1 week) did not mitigate systemic toxicity in the mice. On the other hand, lowering the doses decreased the level of toxicity but it also reduced the treatments effectiveness against B16F10 melanomas. Treatment with anti-CD137/IL-2-Fc caused a dose-dependent systemic cytokine storm resulting in heightened cytokine levels. By conjugating these immune agonists to the surface of liposomes, the researchers were able to eliminate the toxic side effects, indicated by rapid weight loss and elevated cytokine levels, associated with systemic administration of IL-2 and CD137 antibodies. As seen in Figure 10, mice treated with the modified liposome had significantly delayed tumor growth compared to an isotype control liposome (Lipo-IgG) and systemically administered anti-CD137/IL-2-Fc (P < 0.05) and maintained constant body weight which signifies the absence of systemic toxicity. Furthermore, mice that were treated with the immunoliposome had a significantly higher survival percent compared to the untreated group, the free IL-2-Fc/αCD137 treatment group, and the liposome control group (P < 0.001) For more information, a detailed review on liposomes and lipid nanoparticle drug delivery systems was written by Kraft and his colleagues [41].
Figure 10: Therapeutic efficacy of immunoliposome IL-2-Fc/αCD137 therapy. Groups of C57BL/6 mice were inoculated with B16F10 melanoma cells followed by systemic injections of treatment groups on days 8, 10, 12, and 14 post inoculation. Data shows mean tumor sizes (A), relative body weight indicative of systemic toxicity (B), and overall survival of mice (C). Error bars represent mean ± s.e.m. (n = 6-7 per group). Data analyzed using two-tailed Student's t-test (*P < 0.0005, ***P < 0.001). Figure adapted from Ref [40].

One of the most common materials used for fabricating nanoparticles are synthetic polymers due to their ease and affordability of synthesis, wide availability, and customizability. Synthetic nanoparticles made from materials like PLGA and PEG allow for a controlled release of immunomodulatory agents to prolong the effect of the drug while minimizing potential side effects. Synthetic nanoparticles also allow for a modifiable platform to control pharmacokinetic parameters, control biodistribution, and control targeting specificity. In a study done by Schmid et. al., nanoparticles made from FDA-approved PLGA and PEG were designed to target T cells that commonly infiltrate the TME and “hitchhike” to the tumor site before releasing SD-208, a TGFβRI kinase inhibitor [42].
TGF\(\beta\) plays a role in immunosuppression in the TME \cite{77}, so by administering SD-208 the researchers hope to reverse the immunosuppressive TME. To establish the T-cell targeting system, the researchers conjugated anti-PD-1 antibody fragments to the surface of the PLGA/PEG nanoparticle to target CD8\(^+\) T-cells that were PD-1\(^+\) because these T-cells frequently infiltrated the tumor site \cite{78}. To assess the therapeutic potential of the nanoparticle delivery system, the researchers conducted in vivo studies using the MC38 colorectal cancer model (Figure 11). Mice were inoculated with MC38 tumor cells. After 5 days, mice were administered the nanoparticle formulation or free drugs (20\(\mu\)g anti-PD-1 and 40\(\mu\)g SD-208) and received subsequent doses every other day up to a total of 10 doses. Free administration of the drugs had no reductive effects on tumor growth, but tumor growth was delayed when the SD-208 was delivered using the PD-1 targeting nanoparticles. Additionally, the survival rate of mice treated with this nanoparticle formulation was significantly higher compared to the other treatment groups (\(P < 0.001\)). However, tumor growth in mice treated with the forementioned nanoparticle formulation continued to progress until it reached a tumor volume of 2000 mm\(^3\), like the untreated and free drug groups but delayed by 7 days. Even though immune evasion prevailed in this tumor model, the nanoparticle formulation was able to delay growth and offers a platform for further modification to create a synergistic therapy that will utilize the niche of hindered tumor growth.
Figure 11: Therapeutic effect of targeted delivery of SD-208 (a TGFβR1 inhibitor) using anti-PD-1 tagged nanoparticles. C57BL/6 mice were inoculated with MC38 cells and treatment with nanoparticle formulations or free drugs occurred 5 days post inoculation. Mice received subsequent doses every other day up to a maximum of 10 doses. Tumor volume (A) and mice survival (B) was monitored over course of study. Error bars represent mean ± s.e.m. (n = 6). Data analyzed using Mantel-Cox test (***P < 0.001). Figure adapted from Ref [78].

In a subsequent study done by the researchers to repurpose the nanoparticle targeting system, the researchers considered the possibility of eliciting an inflammatory response in the TME by delivering a Toll-like receptor (TLR) 7/8 agonist, R848, to make the MC38 tumor more sensitive to immune checkpoint blockade therapy. The researchers conducted another in vivo study using R848 instead of SD-208 (Figure 12). Mice were inoculated with MC38 tumor cells and received doses on day 5 followed by doses every other day for a maximum of 10 doses. The delivery of R848 using PD-1 targeted nanoparticles significantly delayed tumor growth compared to untreated mice and mice that received free drugs (P < 0.05) and significantly more of the mice treated with the nanoparticles survived at the end of the study (P < 0.001). Synthetic nanoparticles allow researchers to easily
customize the surface and structure of the nanoparticle to optimize immunotherapeutic drug delivery.

Figure 12: Therapeutic effect of targeted delivery of R848 (a TLR7/8 agonist) and sensitization of tumors to PD-1 blockade. C57BL/6 mice were inoculated with MC38 tumor cells followed by nanoparticle or free drug treatment on day 5 and every other day for a total of 10 doses. Tumor volume and animal survival were monitored to assess efficacy (A,B). For graph C-D, nanoparticle or free drug treatment were administered on days 5, 7, and 9 after tumor inoculation to inflame the tumor. Mice were then treated on days 11, 14, and 17 with anti-PD-1 antibody through intraperitoneal injection. Tumor volume and animal survival were monitored to assess efficacy (C,D). Error bars represent mean ± s.e.m. (n = 6-7). Data analyzed using Mantel-Cox test (*P < 0.05, ***P < 0.001). Figure adapted from Ref [78].

Natural polymeric nanoparticles are fabricated using polymers derived from natural compounds. Natural compounds like HA, alginate, and chitosan have the advantage of intrinsic biodegradability and biocompatibility. Drawbacks of using natural polymers
include the variability between batches, potential for contamination, and difficulty to scale synthesis. In a study done by Shi et. al., chitosan nanoparticles with mannose ligands conjugated to the surface were loaded with whole tumor cell lysates (Man-CTS-TCL nanoparticles) to act as a cancer vaccine that targets specific DCs. One of the studies conducted by the researchers assessed the prophylactic capabilities of the nanoparticle vaccine formulation when challenged by an inoculation of $1 \times 10^5$ B16 tumor cells. Vaccination using Man-CTS-TCL nanoparticles delayed tumor growth resulting in a mean tumor volume less than 500 mm$^3$ 21 days after tumor cell inoculation. The tumor volume in mice vaccinated with Man-CTS-TCL was significantly smaller than the tumor volume in untreated mice ($P < 0.05$), in mice vaccinated only by using tumor cell lysates, in mice treated with chitosan nanoparticles lacking mannose ligands ($P < 0.05$), and in mice treated with chitosan nanoparticles with mannose ligands but lacking the tumor cell lysate payload ($P < 0.05$). To further solidify the conclusion that Man-CTS-TCL nanoparticles are an effective vaccination system, the researchers assessed the therapeutic effects of the nanoparticles by vaccinating mice 7 days after receiving subcutaneous injections of B16 melanoma cells. Similar to the prophylactic study, Man-CTS-TCL nanoparticles significantly inhibited B16 tumor growth compared to the untreated group ($P < 0.05$). Nanoparticles made from natural polymers have intrinsic biodegradable and biocompatible properties. Nanoparticles are a highly customizable drug delivery platform that can be used to suit a variety of immunotherapeutic applications ranging from delivering cancer vaccines to acting as artificial APCs.
Drug Conjugates

Drug conjugation is a simple and effective modification strategy to improve the efficacy of systemically administered immunomodulatory drugs. In drug conjugation, immunomodulatory agents are simply conjugated to a targeting ligand, normally a monoclonal antibody, or synthetic polymers to modify the pharmacokinetics of the agents/drugs and minimize their side effects. The two categories of drug conjugates reviewed here are antibody-drug conjugates and polymer-drug conjugates. In cancer immunotherapy, antibodies used alone can recognize specific antigens on or near the tumor site to elicit a cytotoxic response, but the curative effects are limited unless the monoclonal antibodies are modified through conjugation. Antibody-drug conjugates utilize the targeting capabilities of monoclonal antibodies and the cytotoxic/immunotherapeutic effects of the conjugated drug. The basic design of these conjugations consists of the antibody, a linker, and the drug. Any of these three components can be modified to create the best drug delivery system for the intended application. Polymer-drug conjugation allows researchers to modify the pharmacokinetics of immunotherapy drugs, protect the drug from the environment, and allow for the conjugation of targeting moieties. For instance, conjugating immunotherapeutic or cytotoxic drugs to a synthetic polymer such as PEG protects the drugs from enzymatic degradation and rapid clearance via the liver and kidneys. As a result, polymer-drug conjugates generally increase circulation time compared to the parent drug. Polymer-drug conjugates depend on passive accumulation at tumor sites and can be further modified with targeting ligands in order to bind specific immune cell targets or cancer cells. Many classes of polymer-drug conjugates exist including polymers conjugated to biological proteins, small molecule drugs conjugated to
a long polymeric chain, dendrimers, and others. These various conjugate arrangements can elicit different pharmacokinetics and incorporate drug release mechanisms to create the desired therapeutic effect [44]. Conjugating small-molecule drugs to polymer chains offers several advantages such as improved solubility, increased drug stability, prolonged circulation half-life, and altered biodistribution [74]. Conjugating polymers to biological proteins shield the antigenic epitopes through steric hindrance as well as shields the protein from circulating proteolytic enzymes and the MPS. The improved pharmacokinetics of biological proteins reduces the dosage thus improving the safety of treatment. Today, many drug conjugates have been approved by the FDA or are being tested in clinical trials [44,73].

Current advances in oncology research have primarily used antibody- and polymer-drug conjugation to deliver cytotoxic drugs; however, it is theoretically possible to replace the drug component with common immunotherapies in future generations [43,44]. By conjugating drugs to polymers and/or antibodies, researchers achieve a slower clearance rate, prolonged drug circulation, and can potentially alleviate toxic side effects and the risk of autoimmunity associated with systemic immunotherapy.

**Polymer-Drug Conjugates: Design Considerations**

The first rational model for “polymeric prodrugs”, or polymer-drug conjugates, was created by Professor Helmut Ringsdorf in 1975 [45]. These conjugates had five major components: a solubilizer compound, the drug, the polymer backbone, spacers, and a transport/targeting component. Any of these components can be modified to alter the pharmacokinetics and biodistribution of the drugs. The polymer choice for the backbone dictates which drugs can be conjugated, the biocompatibility of the drug conjugate, biodegradation and clearance rate, and drug stability. One of the most important design
considerations is the type of linker between the polymer and the drug. Through the
manipulation of linker chemistry, the release mechanism can be modified to focus drug at
the targeted site. This is especially important in preventing off-target drug release while in
circulation. These linkers can be pH-responsive, degradable by enzymes present at high
levels at tumor sites, or responsive to lysosomal enzymes. For example, Lv et al. conjugated
paclitaxel (PTX), a cancer therapeutic drug, to 3,3’-dithiodipropionic acid functionalized
methoxy poly(ethylene glycol)-b-poly(L-lysine) (mPEG-b-P(LL-DTPA)) to form a
drug conjugate (P(L-SS-PTX)) \[46\]. In this case, the conjugate linker is a
disulfide bond between the carboxyl groups of mPEG-b-P(LL-DTPA) and PTX which is
unstable in reductive and acidic environments. This mechanism allows for drug release
once the drug conjugate is taken into the cell’s endolysosomal pathway. This polymer-drug
conjugate can also be released in the extracellular matrix of the tumor due to the lower pH
and high concentrations of glutathione (GSH). During an \textit{in vitro} drug release study, drug
release from P(L-SS-PTX) was slow, releasing less than 8% of the conjugated PTX, over
the time frame of the study (120 hours) when placed in an extracellular environment that
mimics the TME (pH of 7.4 with a concentration of 20 \textmu M GSH). However, when the
concentration of GSH is increased to 10 mM, P(L-SS-PTX) rapidly releases more than
75% of the PTX over the 120-hour time frame of the study. To simulate the environment
of an endosome, the drug release profile was analyzed in an environment with a pH of 5.
P(L-SS-PTX) released approximately 50% of the PTX payload over 120 hours. Overall,
their drug design barely released any of the cytotoxic drug in neutral environmental
conditions but released a majority of the payload in the acidic and reductive environments.
Additionally, the researchers conducted an \textit{in vivo} antitumor activity study using the B16F1
melanoma tumor model to assess the therapeutic capabilities of P(L-SS-PTX) compared to untreated mice, mice systemically administered PTX, and mice injected with P(L-PTX), a similar polymer-PTX conjugate that lacks disulfide bonds (Figure 13). Mice injected with P(L-SS-PTX) had an average tumor volume of approximately 250 mm\(^3\) which was significantly lower than the tumor volume of mice injected with P(L-PTX) (\(P < 0.01\)) and systemically administered PTX (\(P < 0.001\)). The drug conjugate effectively hindered tumor growth without toxic side effects, as indicated by a lack of weight loss, compared to free systemic administration of paclitaxel and the drug conjugate lacking the disulfide linker. This study did not focus on the use of an immunotherapy; however, it serves as a proof of concept that can be applied to other immunotherapeutic agents.

**Figure 13:** *In vivo* anti-tumor efficacy of P(L-SS-PTX), a polymer-paclitaxel drug conjugate. C57BL/6 mice were inoculated with B16F1 melanoma cells and tumor volume was allowed to grow until it achieved a tumor volume of approximately 20-30 mm\(^3\). Mice were treated with (a) PBS, (b) free PTX, (c) P(L-PTX), or (d) P(L-SS-PTX). Tumor volume (A) and body weight change (B) was assessed for treatment efficacy. Error bars represent mean ± SD (\(n = 6\)). Data analyzed using Student’s t-test (*\(P < 0.05\), **\(P < 0.001\), #\(P < 0.001\)). Figure adapted from Ref [46].
Antibody-Drug Conjugates: Design Considerations

Antibody-drug conjugates have similar design considerations as polymer-drug conjugates; however, antibodies provide a mechanism to target specific cell populations such as tumor cells or tumor leukocytes via receptor-ligand binding. The basic design components of antibody-drug conjugates are the antibody, the drug, and the linker. When considering which antibody to use for the conjugation, researchers should consider an antigen overexpressed on tumor cells (or tumor-associated leukocytes) but not expressed on normal, healthy cells. In a review done by Perez et al., a list of target antigens for various types of cancer is provided [43]. Once an antibody is chosen that will provide specific binding, other properties must be considered such as antibody stability after conjugation, in systemic transit, and at the site of targeted tumor or immune cells. Like polymer-drug conjugates, the linker plays an important role in the stability and drug release of antibody-drug conjugates. These linker components can be sensitive to lysosomal enzymes, pH-responsive, or responsive to glutathione (a reducing agent). Some antibody-drug conjugates utilized non-cleavable linkers. In these cases, the payload can only be released once the conjugate is taken into the cell and the antibody is degraded. After determining the best antibody and linker to suit the application, the site of conjugation onto the antibody is another important consideration as the conjugation site greatly impacts the activity of the drug conjugate. Most researchers use alkylation of reduced interchain disulfides, acylation of lysine residues, or alkylation of genetically engineered cysteine residues to combine the drug and linker to the antibody [47]. Figure 14 provides an overview of the key design components of antibody-drug conjugates. Antibody-drug conjugations gives researchers the ability to target key cells to elicit an antitumor response and warrant further
research to determine effective designs that can deliver immunomodulatory agents to tumors and associated immune cells/organs.

**Figure 14:** Key design components of antibody-drug conjugates. PK, pharmacokinetics. Figure adapted from Ref [43].
Conclusion

The development of immunomodulatory treatments has made tremendous strides since the FDA approval of IFN-α to treat leukemia. Cancer immunotherapy has become a vital asset for oncologists to treat cancer that has proven resistant to typical cancer therapies including radiotherapy and chemotherapy. Immunotherapy offers a less toxic alternative that has long-term curative effects to inhibit cancer growth, metastasis, and recurrence. Though widely successful, many immunomodulatory agents in use are not effective in patients with tumors that lack immunogenicity and can have adverse side effects when used at high doses for long treatment windows. To solve these issues, researchers have developed a wide range of biomaterials that can be used to deliver drugs systemically and locally. By using these immunomodulatory biomaterials, researchers can target specific immune cells and cancer cells, improve the pharmacokinetics and biodistribution of immunotherapies, and improve the therapeutic window of approved and experimental immunotherapies. As a result of the targeted delivery systems, biomaterials effectively use low doses of immunomodulatory drugs such as IL-2, PD-1, or PD-L1 to elicit an effective anticancer immune response without toxic side effects. Biomaterials also offer a modifiable platform for spatiotemporal drug release to promote controlled, long-term, and responsive treatments. Each biomaterial discussed in this review has key features that suit the intended application of cancer immunity treatment. When considering an effective biomaterial for immunomodulation, researchers must investigate which design parameters best suit their intended use. Accordingly, we have reviewed hydrogels, scaffolds, microparticles,
nanoparticles, and drug conjugates. The key design criteria of each biomaterial were discussed, with a main focus on the effect of physical characteristics of the biomaterial on drug delivery and its application. Immunomodulatory biomaterials such as those discussed within this article have the potential to revolutionize cancer immunotherapy and improve patient outcomes.
References


65. Manolova, Vania, Anna Flace, Monika Bauer, Katrin Schwarz, Philippe Saudan, and Martin F. Bachmann. “Nanoparticles Target Distinct Dendritic Cell


75. Turecek, Peter L., Mary J. Bossard, Freddy Schoetens, and Inge A. Ivens. “PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical

