Synthetic immune niches for cancer immunotherapy

_Citation for published version (APA):_

DOI:
10.1038/nri.2017.89

_Document status and date:_
Published: 01/03/2018

_Document Version:_
Accepted manuscript including changes made at the peer-review stage

_Please check the document version of this publication:_

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher’s website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

_General rights_
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:
www.tue.nl/taverne

_Take down policy_
If you believe that this document breaches copyright please contact us at:
openaccess@tue.nl
providing details and we will investigate your claim.

Download date: 20. Mar. 2019
Synthetic immune niches for local control of anti-cancer immunity

Jorieke Weiden¹, Jurjen Tel²,³ and Carl G. Figdor¹

1. Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.
2. Department of Biomedical Engineering, Laboratory of Imunoengeneering, Eindhoven University of Technology, Eindhoven, the Netherlands
3. Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands

Cancer immunotherapy can successfully promote long-term anti-cancer immune responses, although still only a limited number of patients benefit from treatment and at the cost of sometimes severe treatment-associated adverse events. Local immunomodulation may enable more effective treatment at lower dose and at the same time prevent systemic toxicity. Local delivery of engineered three-dimensional scaffolds may fulfil this role by acting as synthetic immune niches that boost anti-cancer immunity. In this Opinion article, we highlight the potential of scaffold-based adoptive cell transfer and scaffold-based cancer vaccines, that although applied locally can enforce systemic anti-tumour immunity. Furthermore, we discuss how scaffold-based cancer immunotherapy may contribute to the development of the next generation of cancer treatments.

Immunotherapy has entered centre stage as a novel cancer treatment modality. A wide variety of promising immunotherapeutic strategies are available, which aim to elicit anti-cancer immunity by generating robust and durable tumour-directed immune responses. Systemic administration of immune checkpoint blocking antibodies that target the co-inhibitory receptors CTLA-4 and PD-1 on T cells has successfully induced remarkable long-lasting survival benefit, although so far this only applies to a small fraction of patients and at the cost of sometimes severe immune-related adverse events¹⁻⁴. In adoptive T cell therapy, the number of circulating tumour-specific T cells is enhanced by systemic infusion of either ex vivo-expanded autologous tumour-infiltrating T lymphocytes, or T cells engineered to express high affinity T cell receptors (TCR) or chimeric antigen receptors (CAR). Encouraging results have been reported for various solid cancer types⁵⁻⁹, but clinical efficacy is hampered by a lack of cell persistence in vivo, poor T cell functionality and insufficient localization of infused lymphocytes at the tumour site¹⁰. A more tolerable strategy to expand the tumour-reactive T cell pool is dendritic cell-based¹¹,¹² or synthetic therapeutic cancer vaccines¹³. Although clinical responses have been observed with these approaches resulting in FDA approval of DC vaccine Provenge by
Dendreon for prostate cancer\textsuperscript{14}, achieving durable clinical responses in established cancers remains challenging. One important factor underlying the limited efficacy and substantial toxicity of current immunotherapeutic strategies is their systemic delivery. For example, intravenous application of immune checkpoint inhibitors necessitates the use of high doses to obtain adequate local concentrations, whereas local administration can achieve powerful systemic anti-cancer T cell responses and decreases the risk of treatment-associated toxicity\textsuperscript{15-17}. Systemic infusion of lymphocytes in adoptive T cell therapy hampers their efficient delivery and commands co-treatment with lymphodepleting chemotherapy and high doses of IL-2, which although imperative for T cell function and survival are highly toxic\textsuperscript{10}.

Conversely, local immunomodulation may provide opportunities for more specific, effective and less toxic treatment strategies that can enforce systemic anti-cancer immunity\textsuperscript{18}. Local immunotherapy initially focused on reverting the immunosuppressive microenvironment of the tumour, as reviewed elsewhere\textsuperscript{19, 20}, and in the tumour-draining lymph nodes (TDLN). Targeting the TDLN is of particular interest as it is the primary immune niche involved in priming and expansion of tumour-reactive T cells, but is also under direct control of the upstream tumour\textsuperscript{21}. In this Opinion article, we will discuss therapeutic interventions that modulate the immunosuppressive state of the TDLN and how these local approaches can confer systemic immunity against cancer. Moreover, we will detail how carefully designed three-dimensional (3D) scaffolds can be exploited as synthetic immune niches. We believe that synthetic immune niches can improve therapeutic benefit of cancer immunotherapy by more precise manipulation of tumour-directed immune responses, whilst simultaneously preventing toxic side-effects through local administration (FIG. 1). We will discuss how 3D biomaterial-based scaffolds may enforce systemic anti-tumour immunity via delivery of \textit{ex vivo} trained immune cells or \textit{in situ} re-programming of host cells, and we will illustrate how these novel strategies may contribute to the development of the next generation of cancer immunotherapies.

\textbf{Modulating natural immune niches}

Tumours employ a wide variety of mechanisms to escape immune surveillance in a process termed cancer immunoediting\textsuperscript{22}, including the production of immunomodulating factors that impair immune cell priming, repress initiated immune responses and recruit suppressive immune cells instead of effector T cells\textsuperscript{22-24}. Lymph drainage of these factors shifts the TDLN to an immunosuppressive state, which is detrimental for the interaction between tumour antigen-loaded dendritic cells (DCs) and naive T cells\textsuperscript{21}. Therefore, it will negatively affect the ability of DCs to mount robust CD8\textsuperscript{+} cytotoxic T cell (CTL) responses accompanied by strong
Th1-polarization, both pivotal for effective anti-tumour immunity\textsuperscript{25}. An improved understanding of the tolerogenic environment within TDLN in various types of solid cancers\textsuperscript{26-29} and its role in creating systemic tolerance\textsuperscript{21,30,31} triggered the development of interventions aimed at reverting the immunosuppressive state of TDLN. This was further substantiated by studies showing superior CTL responses in mice where antigen and adjuvant-loaded nanovaccines were directed to TDLN, suggesting that the tumour antigen-experienced state of TLDN may be exploited despite its immunosuppressed state\textsuperscript{32,33}. Consequently, immunostimulatory compounds were applied to boost priming and effector functions of CTLs by for instance supplementing toll-like receptor (TLR) ligands\textsuperscript{34}. Systemic delivery of immunostimulants failed to obtain clinical efficacy due to highly toxic side effects\textsuperscript{18}, emphasizing the need for local administration.

Clinical studies tested intradermal injection of CpG oligodeoxynucleotides (ODNs), that bind TLR-9, alone or together with cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) around the primary tumour excision site of early stage melanoma patients\textsuperscript{15,36}. The combination was found to be superior in activating various DC subsets within TDLN, which coincided with an increased frequency of melanoma-specific CTLs and significantly reduced the occurrence of lymph node metastases\textsuperscript{36}. Similarly, local peritumoural injection of oncolytic viruses able to infect and kill tumour cells and at the same time produce GM-CSF can lead to systemic anti-tumour CTL responses in melanoma patients\textsuperscript{37,38}. Clinical studies combining local administration of CpG ODNs with radiotherapy also demonstrated induction of systemic anti-tumour effects\textsuperscript{39,40}. Furthermore, studies in animal models have shown that local and slow release of CTLA-4 blocking antibodies\textsuperscript{15-17}, agonistic antibodies against the co-stimulatory receptors CD40\textsuperscript{41,42} or others\textsuperscript{43} drive potent CTL responses and delay tumour growth. Notably, local administration of low doses of immune checkpoint blocking antibodies were equally effective when compared to high dose systemic administration in inducing systemic anti-tumour immunity and immunological memory. Moreover, local immunotherapy avoids high serum antibody levels associated with systemic delivery, thereby limiting systemic non-specific T cell activation and inflammation\textsuperscript{15-17,41,42} and reducing toxicity.

Rather than applying general immunostimulants that evoke broad immune cell activation, priming of tumour-specific CTLs requires providing DCs with synthetic tumour antigens, DC-activating adjuvants and pro-inflammatory cytokines (reviewed in\textsuperscript{37,44}). These may be delivered through bolus injection or can be co-presented by solid phase polymer-based, lipid-based or inorganic nanovaccines. Functionalization of nanoparticles with molecular ligands or tuning their net charge\textsuperscript{45} allows for selective targeting of cargo to the tumour\textsuperscript{46}, draining lymph
nodes, or specific cell populations residing within TDLN\textsuperscript{47, 48}. Alternatively, T cells may also be directly activated by artificial antigen presenting cells (APCs) - nanovaccines that mimic DCs in their antigen-presenting and T cell-priming function by presenting T-cell stimulatory ligands\textsuperscript{49}. Thus, nanovaccines are especially useful to co-deliver multiple signals to immune cells in a tightly regulated manner at specific sites.

Although strategies to reprogram immunosuppressed TDLN may evoke systemic immune responses, the continuous immunosuppressive state of the tumour microenvironment and TDLN may still prove insufficient to unleash the full potential of tumour-reactive CTLs and therefore warrants investigation of alternatives. The use of biomaterial-based scaffolds to boost the anti-tumour response is particularly promising as it not only provides opportunities for sustained delivery of immunomodulators or cells at a specific location with spatiotemporal control, but also accommodates the establishment of a permissive immunogenic microenvironment.

**Design of synthetic immune niches**

Engineered scaffolds with defined chemical, mechanical and physical properties create new opportunities for local cancer immunotherapy. They form the building blocks of synthetic immune niches, which are 3D biomaterial-based scaffolds that mimic natural lymph nodes and provide a localized alternative site for immune cell interaction, expansion and dispersion. Thereby, these synthetic immune niches can be exploited to modulate the (anti-cancer) immune response and circumvent immunosuppressed TDLN. Re-programming the anti-tumour immune response at an alternative location is reminiscent of extranodal immune cell activation that takes place in naturally occurring tertiary lymphoid structures (TLS) surrounding tumours. Notably, a high density of peritumoural TLS correlates with a higher Th1 and CD8\textsuperscript{+} memory T cell-oriented infiltration within the tumour\textsuperscript{50} resulting in a significant favourable prognosis for almost all human cancers that harbour TLS\textsuperscript{51}.

The physical space provided by synthetic immune niches is essential for their function. Scaffold porosity ensures diffusion of nutrients and chemical cues throughout the matrix\textsuperscript{52}, which is important for cell survival and regulation. Moreover, the interconnected porous architecture of the material provides space to encapsulate cells to create a depot of expanding immune cells, or allows incoming leukocytes \textit{in situ} to interact with molecular signals decorated onto the scaffold. As such, 3D scaffolds can modulate tumour-directed immune responses in two different manners: either by acting as a cellular delivery vehicle of potent immune cells together with immunomodulatory agents (FIG. 2), or as an engineered microenvironment that creates a
depot of activating ligands for incoming immune cells (FIG. 3). To function as synthetic immune niches, scaffolds must fulfil the following criteria: 1) structural rigidity to withstand tissue pressure, 2) porosity to accommodate in- and out-flux of immune cells, 3) spatiotemporal control over signalling cues to modulate immune cell function. Synthetic immune niches are particularly promising as they not only enable local immunomodulation in an immunogenic context, but also address limitations of current cancer immunotherapeutic strategies related to localized cellular delivery and sustained availability of immune stimulants. Another advantage of synthetic immune niches is that through careful design the release profiles of molecular and cellular cargo can be fine-tuned at high spatiotemporal resolution (BOX 1).

**Scaffold-based adoptive cell transfer**

Adoptive transfer of *ex vivo*-activated blood DCs pulsed with tumour peptides or expanded tumour-reactive T cells are promising immunotherapeutic strategies to generate protective anti-tumour immune responses. However, challenges remain with respect to insufficient tumour localization, impaired cell survival, the need to deliver large numbers of cells and toxicity as a result of co-administered drugs\(^10,53,54\). We propose that delivering cells within 3D scaffolds is a powerful tool to localize immune cells to a specific site and provide them with additional cues to enhance their survival, activation and proliferation, followed by their continuous release into the environment (FIG. 2). Initial evidence for the validity for this approach was given using alginate-based hydrogels that self-gelate *in situ* for local delivery of *ex-vivo* activated DCs together with IL-15 superagonist (SA), to promote CD8\(^+\) T cell recruitment\(^55-57\). Subcutaneous injection of alginate hydrogels restricted tumour growth of established melanomas through local accumulation of a population of tumour-specific CTLs\(^55,56\). This strategy proved more effective than injection of DCs and IL-15 SA alone, underlining the additive value of this 3D scaffold. Moreover, since *ex vivo* culturing of DCs is a laborious process, the authors tested delivery of alginate gels containing CpG ODNs and IL-15 SA without any DCs to the peritumoural site and observed comparable suppression of tumour growth\(^56\). In other mouse studies, delivery of DCs in macroporous fibrin gels significantly outperformed injection of free DCs with respect to delaying tumour outgrowth\(^58\). DCs were found to interact with CTLs that infiltrated the scaffolds, suggesting immune priming took place within the engineered niche. Interestingly, this approach resulted in systemic anti-tumour activity as it provided protection against tumour re-challenge.

The delivery of tumour-specific T cells was initially explored using biodegradable PEG-g-chitosan temperature sensitive hydrogels that gelate *in situ* upon injection\(^59\). Antigen-specific
CTLs could readily traffic through the hydrogel without losing their killing capacity. Another study using a different type of thermosensitive chitosan-based hydrogels found that mechanical properties, gelation behaviour and porosity of the scaffold are highly important for T cell survival, ensuring cellular proliferation and dispersion. Importantly, gels containing pores of 50 to 500um in size optimally facilitated T cell escape towards the tumour, and maintained their ability to kill tumour cells. Scaffold-based adoptive transfer of T cells was further explored by creating reservoirs of potent tumour-specific CTLs using macroporous alginate scaffolds decorated with adhesion peptides. To promote activation and proliferation of encapsulated T cells, microparticles containing IL-15 SA and coated with αCD3, αCD28 and αCD137 agonistic antibodies were incorporated. Upon scaffold transplantation in different mouse tumour models, the residing and proliferating CTLs maintained a non-exhausted phenotype. Whilst this approach successfully prevented tumour outgrowth even when an immunosuppressive tumour microenvironment was present, direct injection of pre-stimulated CTLs without scaffold only gave a modest survival advantage.

Together, these studies indicate that exploiting scaffolds as cellular delivery vehicles for adoptive cell therapy to expand DCs and CTLs in situ effectively boosts cell persistence and at the same time dictates and maintains cellular localization. We are convinced that in situ instruction and training of immune cells, as exemplified above, might greatly improve current protocols for ex vivo immune cell expansion.

**Scaffold-based cancer vaccines**

In addition to exploiting 3D scaffolds to create reservoirs of immune cells, we strongly believe that synthetic immune niches are excellent vaccine delivery vehicles. They guarantee localized and prolonged availability of multiple immunomodulatory factors in a spatiotemporal controlled manner. Moreover, the supplied matrix can be designed to attract cells in vivo and provide a local space where incoming DCs can take up tumour antigen in a controlled pro-inflammatory environment (FIG. 3).

Recent evidence indeed demonstrates successful presentation of immunomodulatory molecules at a localized site to where DCs are mobilized. Elegant studies explored the use of a two-step hybrid strategy, where initially DCs are recruited towards the matrix by engineering mPEG–PLGA hydrogels that release GM-CSF. Next, viral and non-viral vectors carrying antigen and adjuvants were injected close to the gel. Importantly, DCs initially recruited towards the scaffold became activated and effectively migrated out of the scaffold towards the draining lymph node, leading to IFNγ production by CTLs. This two-step vaccine could
significantly impair tumour growth both in prophylactic and therapeutic melanoma tumour models. A more straightforward system was engineered by the Mooney lab, which designed a macroporous PLG matrix\textsuperscript{63, 64}. Notably, whereas GM-CSF was released in a sustained manner from the scaffold facilitating DC recruitment, CpG and tumour lysate were immobilized onto the matrix and therefore provided continuous stimulation to incoming DCs. Implantation of this vaccine carrier induced a profound expansion of antigen-specific CTLs, resulting in a significant delay in tumour growth and enhanced survival in preclinical melanoma\textsuperscript{63, 65} and glioma models\textsuperscript{64}. This approach proved to be highly flexible as an array of different chemokines, cytokines and adjuvants can be incorporated\textsuperscript{66, 67}. This PLG-based scaffold vaccine is currently tested in a phase I clinical trial for metastatic melanoma\textsuperscript{68} (NCT01753089). To overcome the cumbersome implantation of PLG matrices, Mooney and colleagues also explored biodegradable mesoporous silica rods as vaccine-delivery vehicles, which spontaneously self-assemble into a 3D scaffold upon subcutaneous injection\textsuperscript{69}. Effective accumulation of mature APCs in the scaffold was observed, resulting in high numbers of tumour antigen-specific CTLs effectively delaying tumour growth. Pre-formed alginate cryogels form another group of attractive biomaterials. They are injectable as they collapse due to shear stress during injection, but rapidly regain shape once in the body\textsuperscript{70}. Structure, mechanical strength and localization of these cryogels can be more precisely controlled than for instance in situ gelating systems\textsuperscript{71}. Engineered macroporous cryogels containing GM-CSF, CpG ODNs and irradiated tumour cells facilitated influx of DCs and promote uptake of tumour antigen in an immunogenic context, resulting in long term protective anti-tumour immunity\textsuperscript{72}. Combining these delivery systems with molecules that can modulate the immunosuppressive tumour microenvironment may further enhance priming and effector functions of tumour-specific immune cells. Hence, a dextran-based injectable hydrogel was engineered, which slowly releases DC-recruiting chemokine CCL20 and presents microparticles containing IL-10 siRNA and lymphoma plasmid DNA antigens\textsuperscript{73, 74}. These multi-component immunomodulating immune niches provoked a Th1-oriented and strong CTL response, resulting in long-term tumour protection\textsuperscript{73, 74}. Taken together, these studies elegantly show that while scaffold vaccines effectively restricted tumour growth, bolus injection of components without a scaffold appeared to be ineffective\textsuperscript{63, 65, 69, 72}. This clearly demonstrates the potential of synthetic scaffolds for in situ modulation of immune responses, yet resulting in systemic anti-cancer immunity. More insight into the importance of immune cell trafficking in and out of 3D scaffolds, timing and sustained presentation of chemokines and immunomodulators is required to optimize these approaches.
**Engineering next generation immunotherapies**

The multidisciplinary research discussed above underpins the importance of collaborative efforts between material engineers, chemists and immunologists to fully exploit the currently available and expanding toolbox of 3D biomaterial-based scaffolds. Synthetic immune niches will greatly improve current strategies for cancer immunotherapy in many aspects provided that they consist of 1) the right scaffolding materials, 2) the essential immunostimulatory cues, and 3) work in a highly spatiotemporally controlled manner to support the different phases of the immune response, eventually resulting in systemic anti-cancer immunity.

Several parameters need to be considered to design optimally functioning synthetic immune niches. First, it is important to establish the preferred location of synthetic immune niches with respect to the immunological response. Immune responses initiated in peritumoural TLS seem to be associated with superior tumour-control\(^{50,51}\), suggesting that robust immune priming can take place in vicinity of the tumour, irrespective of the presence of tumour-derived immunosuppressive factors. Since so far most preclinical mouse models used in the work discussed here do not take tumour immunosuppression into account, research is needed that uses more sophisticated mouse models to examine the influence of tumour-derived immunosuppressive factors on the efficacy of scaffold-based immunotherapy in relation to the site of administration.

Another important strength of re-programming anti-cancer immune responses using synthetic immune niches is the flexibility of this approach. Many tools are available to incorporate an array of molecular agents and drugs into scaffolds with varying release profiles (BOX 1). This might for example be exploited when loading scaffolds with *ex vivo* expanded CTLs, by incorporating cytokines such as IL-2 to promote T cell survival. A further extension of scaffold-based immunotherapy might focus on engineering immune niches that not only enhance CTL responses, but simultaneously counteract tumour-induced immunosuppression. For example, by combining approaches discussed above with sustained delivery of immune checkpoint blocking antibodies\(^{65,75}\), agonistic antibodies against co-stimulatory receptors\(^{76-78}\), immunostimulatory factors such as IFNα or TGF-β inhibitors\(^{79,80}\) and/or siRNAs\(^{81-83}\) released from the scaffold or from nanovaccines incorporated into the scaffolds. Finally, scaffolds may support lymphoid neogenesis to simulate peritumoural TLS via delivery of lymphoid tissue inducer stromal cells\(^{84,85}\), combinations of chemokines and cytokines\(^{86}\), or molecular ligands\(^{87}\) that induce lymphoid tissue formation.
Obviously, the challenge is to make the right choices as the number of combinations is almost endless. This starts already with choosing which scaffold to use and how to decorate it, which will largely depend on what the ultimate goal is: expanding immune cells *in-vivo*, stimulating efflux of immune cells, attracting DCs for vaccination purposes, or slowly releasing immune checkpoint inhibitors, to mention a few. In addition, the immobilization strategies are of utmost importance to control temporal release of biomolecules (BOX 1). Most studies discussed here resort to slow release of non-covalently attached biomolecules. It will be particularly interesting to compare various incorporation strategies including covalent coupling of immunomodulators to study the influence of release profiles and force sensing on immune cell activation. Covalent binding of biomolecules may particularly improve the sustained availability of immunomodulatory signals and prevent systemic exposure by dictating their localization\textsuperscript{76, 77}, thereby increasing both efficacy and safety. Future development of advanced methods to exert control over the incorporation and (conditional) release of immunomodulators at high spatiotemporal resolution in 3D scaffolds may result in specific spacing of biomolecules to ensure optimal signalling, e.g. taking the complex clustering of immunomodulating molecules at the immunological synapse into consideration, or releasing molecules covalently linked to scaffolding materials under the influence of light or proteolytic cleavage. Such approaches are especially interesting when scaffolds will be exploited to act as artificial APCs to directly prime incoming T cells using covalently-attached T cell-stimulating ligands. Dissecting the basic mechanisms underlying the multitude of interactions within the immune system and the interaction of immune cells with their microenvironment is critical to advance the field of scaffold-based cancer immunotherapy and gain more control over anti-cancer immunity. Precise manipulation of the defined properties of synthetic biomaterials can expose these processes and therefore synthetic constructs will also need to be used as quantitative tools to study complex immunological processes\textsuperscript{88}.

**Hurdles towards clinical translation**

To exploit the full potential of engineered synthetic immune niches and to facilitate clinical translation, several challenges will need to be addressed in the upcoming years. We feel that this novel field can particularly benefit from lessons learned in regenerative medicine with respect to translating biomaterial-based scaffolds to the clinic\textsuperscript{89}.

One important issue relates to the biocompatibility and degradation behaviour of biomaterial-based scaffolds. Even though the likelihood of systemic toxicity is limited as scaffolds are applied locally, confined acute toxicity and inflammation may still arise in response to
biomaterials or factors dispersed from the scaffolds. Moreover, chronic inflammation and immune activation may occur depending on scaffold composition and degradation behaviour. After the first proof-of-principle studies demonstrated efficacy, the long-term safety of different types of scaffolds with specific combinations of bioactive molecules must be carefully examined. Another principal issue is to confirm the efficacy of synthetic immune niches in pre-clinical mouse tumour models that recapitulate clinically observed tumour-induced tolerance, metastasis, and tumour heterogeneity.

Importantly, regulatory and ethical issues need to be addressed early on when developing synthetic immune niches. This starts with preferentially exploiting GMP clinical grade materials in formulations already approved for clinical use, as it is easier to obtain clinical approval for modifications on existing systems\textsuperscript{89}. Clinical translation is especially challenging as it often involves complex scaffolds that release multiple biomolecules and/or deliver autologous cells. We expect that cell-free scaffold-based cancer vaccines will move to the clinic first due to fewer regulations and relative simpler application, which present well-defined proteins and chemokines to the immune system in situ, similar to a current clinical trial applying PLG-based cancer vaccines\textsuperscript{68}. Concurrently, the translation of functionalized scaffolds incorporating immune cells and/or immunomodulating signals will continue.

**Conclusions**

In this Opinion article, we propose that biomaterials-based 3D scaffolds hold great promise in overcoming restrictions related to limited efficacy and systemic toxicity associated with current immunotherapeutic strategies for cancer. The early work summarized here displays the unprecedented potential of scaffold-based adoptive cell therapy and scaffold-based cancer vaccines to impede tumour growth at a systemic level whilst preventing side effects through local administration. We believe that combining these approaches with counteracting tumour-induced immunosuppression is especially promising to enforce systemic anti-cancer immunity, which is of particular relevance when treating patients in a metastatic setting.

Main challenges in the upcoming years include not only optimizing the design and location of 3D biomaterials but also controlling the spatiotemporal distribution of immunomodulating cues, as the timing of signals to steer immune cell recruitment, activation and proliferation is critical to the immunological response. This will require the development of methods to release or activate immunomodulators from these scaffolds in a spatiotemporally controlled manner. To facilitate clinical translation of synthetic immune niches, it is important to consider key design principles with respect to scaffold composition, degradation behaviour and
functionalization from the start on and investigate in depth the safety of these approaches. Multidisciplinary efforts from both materials sciences, chemistry and immunology are essential to achieve these goals.

Box 1. Tools to engineer synthetic immune niches

Synthetic immune niches are created from natural or synthetic biomaterials such as polymers, lipids or self-assembled structures. Various engineering tools are available to design scaffolds with defined physical, chemical and spatiotemporal characteristics which will influence the immunological response that may be expected, as extensively reviewed elsewhere\textsuperscript{90, 91}. The choice and formulation of a scaffold will dictate its structural integrity, rigidity, porosity and degradation behaviour. Structural integrity and rigidity are essential to provide structural support to promote cell activation and interaction. Scaffold porosity is important as it dictates cellular in- and outflux and influences the surface area with which cells can interact. Pores ranging from 100 to 500um optimally ensure diffusion of nutrients and chemical cues throughout the matrix\textsuperscript{52}.

Scaffolds may be pre-formed and delivered via implantation\textsuperscript{58, 61, 63}, or formulated such that they are injectable and gelate \textit{in situ}, for instance in response to temperature\textsuperscript{59, 60, 62} or by addition of crosslinkers\textsuperscript{55-57}. Minimal invasive delivery through injection is highly favourable, although pre-formed scaffolds enable more control over mechanical structure and localization of the scaffold, and prevent leakage of injected scaffold into the environment as might be the case for \textit{in situ} gelating systems\textsuperscript{71}. Alternatively, pre-formed scaffolds may be designed such that they can be compressed to ensure injectability, and regain shape after injection\textsuperscript{71, 72}.

The strategy by which biomolecules such as chemokines, immune activating ligands or antibodies are incorporated within the scaffold is critical to determine the spatiotemporal release profiles of these agents. First of all, the molecular size of a compound will influence the rate of diffusion through the matrix and into the environment. When compounds are incorporated into scaffolds via physical entrapment, the degradation behaviour of the material will further dictate its release. Immunomodulators may also be incorporated through non-covalent hydrophobic or ionic interaction, in which case hydrophobicity and charge of both scaffold and biomolecules will affect cargo release. Finally, covalent coupling of biomolecules by for instance bio-orthogonal click chemistry enables their prolonged availability until the scaffold disintegrates. Since often multiple incorporation strategies will work in a concerted fashion to determine the release profile of biomolecules, incorporation strategies of the desired biomolecules should be carefully considered in the early stages of designing
immunomodulatory scaffolds and experimentally tested to gain spatiotemporal control over the anti-tumour immune response.

<table>
<thead>
<tr>
<th>Choice and formulation of material</th>
<th>Method of administration</th>
<th>Integration of biomolecules</th>
<th>Incorporation strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocompatibility</td>
<td>Implantation</td>
<td>Chemokines Adhesion molecules Activating ligands Antibodies Cytokines Lymphogenic factors</td>
<td>Physical entrapment Ionic interaction Hydrophobic interaction Covalent coupling</td>
</tr>
<tr>
<td>Biodegradability</td>
<td>Injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural integrity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porosity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rigidity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Acknowledgements**

The authors thank A.B. van Spriel for critically reviewing the manuscript. This work was supported by Institute of Chemical Immunology grant 024.002.009. CF is recipient of the NWO Spinoza award, ERC advanced grant PATHFINDER (269019) and KWO grant 2009-4402 of the Dutch Cancer Society.

**Competing interests statement**

The authors declare no competing interests.

**Author biographies**

Jorieke Weiden received her M.Sc. degree in Biomedical Sciences cum laude from the Radboud University in Nijmegen. She performed internships at Newcastle University, the Netherlands.
Cancer Institute and the Radboud Institute for Molecular Life Sciences. Jorieke is currently working as a Ph.D. candidate developing biomaterial-based scaffolds to modulate tumour-specific T cell responses at the department of Tumor immunology in the Radboud University Medical Center.

Dr. Jurjen Tel is an assistant professor in Immunoengineering. He received his PhD cum laude in 2013 from the Radboud University Nijmegen. Funded by a NWO-Veni grant (2013), he performed his postdoctoral research in the labs of Prof. Carl Figdor and Prof. Wilhelm Huck. Thereafter, he took up a position in the Department of Biomedical Engineering at the Eindhoven University of Technology to lead the new group in Immunoengineering with a focus on decoding cellular interactions exploiting microscale tools.

Prof. Carl Figdor is heading the department of Tumor Immunology at the Radboud Institute for Molecular Life Sciences, located within the Radboud University Medical Center. He obtained his PhD at the Netherlands Cancer Institute, and focused throughout his career on tumor immunology and to translate basic findings towards the clinic. He is one of the founding fathers of the Institute for Chemical Immunology (http://chemicalimmunology.nl/en), aimed at integrating both disciplines, which is also of eminent importance to successfully develop synthetic immune niches.
References


Figure 1. Synthetic immune niches act locally to control the anti-tumour immune response. Current immunotherapeutic strategies are often delivered intravenously, resulting in systemic exposure (indicated in red) and treatment-associated toxicity. This includes cellular immunotherapies that deliver ex-vivo-expanded immune cells (dendritic cell (DC) vaccination, adoptive T cell therapy using tumour-infiltrating lymphocytes (TIL) or chimeric antigen receptor (CAR) engineered T cells), or in vivo acting nanovaccines, immune checkpoint inhibitors and cytokines. On the other hand, local administration may result in more effective treatment at lower dose while at the same time preventing systemic toxicity. Applying synthetic immune niches for scaffold-based adoptive cell transfer and scaffold-based cancer vaccination not only enables local immunomodulation but may also overcome other limitations of current immunotherapeutic interventions related to cellular delivery and sustained availability of immunostimulatory agents.
Figure 2. Scaffold-based adoptive cell transfer. Scaffolds are loaded *ex vivo* with antigen-loaded activated dendritic cells or pre-stimulated tumour-specific T cells. Stimulatory agents can be included to support cell survival, activation and expansion. After administration of the matrix close to the tumour site, potent immune cells proliferate within the scaffold and are released continuously into the tissue environment.
Figure 3. Scaffold-based cancer vaccines. Scaffolds are designed to locally recruit immune cells through the release of chemoattractants. Incoming immune cells are (re-) programmed in situ through the stimulatory signals that they encounter within the scaffold such as antigens and adjuvants, thereby generating mature antigen-loaded DCs. Subsequent T cell priming can occur within the scaffold or by DCs that migrate out of the scaffold towards the draining lymph node.