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Engineering the pre-metastatic niche

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Abstract

The pre-metastatic niche — the accumulation of aberrant immune cells and extracellular matrix proteins in target organs — primes the initially healthy organ microenvironment and renders it amenable for subsequent metastatic cell colonization. By attracting metastatic cancer cells, mimics of the pre-metastatic niche offer both diagnostic and therapeutic potential. However, deconstructing the complexity of the niche by identifying the interactions between cell populations and the mediatory roles of the immune system, soluble factors, extracellular matrix proteins, and stromal cells has proved challenging. Experimental models need to recapitulate niche-population biology *in situ* and mediate *in vivo* tumour-cell homing, colonization and proliferation. In this Review, we outline the biology of the pre-metastatic niche and discuss advances in engineered niche-mimicking biomaterials that regulate the behaviour of tumour cells at an implant site. Such oncomaterials offer strategies for early detection of metastatic events, inhibiting the formation of the pre-metastatic niche, and attenuating metastatic progression.

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B.A.A., G.G.B., and L.D.S. wrote and edited the manuscript. B.A.A. prepared figures. B.A.A. and G.G.B. prepared tables. S.S.R. and J.S.J. edited and provided important consultations for the manuscript.

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1. Introduction

The hypothesis that tumour cells exhibit preferences when metastasizing to organs dates to 1889, when Steven Paget posited in his 'seed-and-soil' hypothesis that the spread of tumour cells is not random but governed by regulated processes and is pre-determined[1]. For example, in breast cancer, metastases tend to form primarily in bone, liver, lung, and brain tissues, which indeed indicates a tropism for specific microenvironments[2]. This 'primed' microenvironment, also known as the pre-metastatic niche (Box 1 and Fig. 1), is involved in promoting tumour cell homing, colonization and subsequent growth at the target organ. Once metastases form at niche sites, the clinical conversation typically changes from curative treatments to the prolongation of progression-free survival. Complications from metastasis are ultimately responsible for 90% of cancer-associated deaths[1,2].

Box 1

The Pre-Metastatic Niche

Kaplan *et al* first described the formation of a pre-metastatic niche mediated by VEGFR1$^+$ bone marrow-derived hematopoietic progenitor cells[4]. They also found that in addition to the arrival of VEGFR1+ BMDCs, TSFs increase the proliferation of fibroblast-like stromal cells, which contribute to local deposition of fibronectin. VEGFR1+ niche cells express VLA-4 that binds to fibronectin and allows them to assemble at the site. Most notably, the VEGFR1+ niche cells act as harbingers of organ-specific carcinoma spread. This study was the first demonstration of a microenvironment designed to attract tumour cells to a target organ, and set the stage for future work to discover additional factors that contribute to niche formation.

Different types of metastasizing cancers have preferences for specific organ targets, implying that certain types of cancer are more likely to migrate to and flourish in specific microenvironments [132–134]. Metastatic breast cancer cells often populate metastatic niches located at the lungs[135], liver[136], brain[137], bone[138], and lymph nodes[139], with each tissue featuring various characteristics that promote tumour cell homing, adhesion, and growth. Aberrantly accumulated proteins produced by tumour-subverted stroma (including organ fibroblasts and endothelial cells) such as fibronectin, collagen IV, tenascin, and periostin promote tumour cell adhesion at metastatic sites[107,140]. Recently, exosomes from pancreatic ductal adenocarcinomas were shown to promote liver pre-metastatic niche formation and increase metastatic burden, demonstrating a role for exosomes in establishing the niche[13]. Additionally, macrophage-like Kupffer cells present at the liver uptake exosomes and subsequently increase TGF-β and fibronectin expression to recruit BMDCs. The ability for exosomes to interact with resident cells to determine the organotropism at target organs was further demonstrated with specific integrins shown to enable tissue targeting[14].

The relative importance and interplay between players of the pre-metastatic niche have yet to be fully understood. This paucity of knowledge is partially due to the young age of the field; however, a significant challenge is posed when attempting to modify the pre-metastatic or metastatic site without experiencing off-target effects. Implanted biomaterials provide an ectopic location that enables deconstruction of the individual

cues leading to pre-metastatic niche formation, tumour cell homing, colonization, and proliferation.

The pre-metastatic niche consists of a complex microenvironment that includes inflammatory immune cells, stromal cells, extracellular matrix (ECM) proteins, tumour-secreted exosomes, and homing factors. Tumour-secreted factors and tumour-derived exosomes (Fig. 1a) mobilize and recruit bone-marrow-derived cells (BMDCs) to niches in secondary organs (Fig. 1b), where they interact with the local stroma to create permissive and attractive sites for metastatic cells (Fig. 1c, d)[3]. The arrival of VEGFR1+ BMDCs to the pre-metastatic site preceded and predicted the arrival of tumour cells[4]. Other BMDC populations that have also been implicated in the formation of the pre-metastatic niche include CD11b+ myeloid cells, myeloid derived suppressor cells (MDSCs), neutrophils, tumour-associated macrophages, and regulatory T cells[5–12]. Tumour-secreted factors and exosomes can also directly modify the host stroma to establish a supportive microenvironment[13,14]. Additionally, fibroblasts, endothelial cells and lung epithelial cells have been associated with the establishment of the pre-metastatic niche via secretion of inflammatory cytokines and chemokines[6,9,15]. The compelling evidence that pre-metastatic niche formation is required for metastases (Box 1) has prompted biologists and biomedical scientists to elucidate the individual and combinatorial cues that affect cell-niche behaviour, with the ultimate aim of developing effective therapeutic interventions.

Because of the complex molecular pathways promoting metastasis, and their overlap with primary tumour progression, the study of the relative contributions of each pathway *in vivo* has been challenging. Strategies based on engineered biomaterials have enabled the deconstruction of these complex environments and the study of distinct processes such as primary tumour formation[16–18], invasion[19], and extravasation[20,21], as well as metastatic cell homing[22], colonization[23] and proliferation[24]. Studying these processes by using engineered ectopic sites *in vivo* can therefore provide key information that can ideally complement insights obtained by genetic modification of the tumour or the host (Table 1). Moreover, the design of artificial biomaterials that mimic the pre-metastatic niche opens up translational opportunities, such as the diversion of metastatic cells away from target organs and the development of early detection strategies[25,26] that had been unattainable with conventional approaches[27].

Here, we review strategies for the design and implementation of engineered biomaterials as pre-metastatic niche mimics. We discuss the choice of synthetic or natural materials, the fabrication method, the inclusion of bioactive cues, and material properties such as degradability and porosity, and examine how biomaterials have been used to probe tumour-cell recruitment to an engineered niche and tumour cell behaviour upon arrival to the niche. We also describe how engineered niches may be used as novel detection and therapeutic strategies.

2. Cancer Cell Recruitment to an Engineered Niche

Cancer cells migrate from a primary tumour to a secondary target organ via a progressive cascade of events, including microenvironmental remodelling processes at each stage of

disease progression[2,28–30]. Following the degradation of the tumour basement membrane, cells invade and gain access to the vasculature to become circulating tumour cells (CTCs)[31]. CTCs respond to chemokine gradients and "home" toward niche microenvironments at a target organ by escaping the vasculature via a process known as extravasation, at which point it is classified as a disseminated tumour cell (DTC)[2,30]. DTCs may be capable of adhering and colonizing the site, provided they have access to a permissible niche.

Tissue engineering approaches have been used to create biomaterial platforms that mimic properties of the pre-metastatic niche (Table 2). Material options include synthetic degradable materials (e.g. poly(lactic-co-glycolic acid), PLG), synthetic non-degradable materials (e.g. polyacrylamide), and natural materials (e.g. silk)[32]. Each of these materials can be formed into a porous scaffold structure that supports the retention of loaded factors or cells, integrates within a host tissue upon implantation, facilitates the formation of a defined microenvironment *in vivo,* and provides an ectopic site for the recruitment of metastatic tumour cells. The choice of material depends on the desired application and feature of the pre-metastatic niche to mimic. For example, in applications where the desired goal is to simulate the bone microenvironment, relatively stiff biomaterials with similar mechanical properties to bone may be advantageous[33]. These materials can be combined with factors to model the properties of the target organ[34] and evaluate the contribution of each factor during homing and colonization[27,35].

2.1. Immune cell trafficking

Immune cells such as MDSCs[36,37], macrophages[38], T-cells[39,40] and monocytes[11] all contribute to niche formation and tumour cell homing. For instance, hypoxic tumour cells secrete lysyl oxidase which crosslinks collagen IV in the lung and facilitates the accumulation of CD11b+ monocytes for niche formation[6,41]. Purified populations of hematopoietic stem and progenitor cells (HSPCs) were also tracked *in vivo* using an orthotopic E0771 adenocarcinoma breast tumour model, and shown to differentiate readily into immunosuppressive myeloid cells[7]. Once immune cells accumulate at distal organs, they secrete a multitude of factors, facilitating the subsequent recruitment and colonization of DTCs[42,43]. Intravital imaging has been used to show the real-time interactions between immune cells and DTCs undergoing colonization, further elucidating the role of myeloid cell populations in providing a primed harbour for tumour cells at target organs[44]. While these studies identify the importance of immune cells in the pre-metastatic niche, few studies have investigated the interplay between tumour and immune cells within the niche itself, at least in part due to a lack of suitable research tools.

The host response to an implanted biomaterial includes several blood-material interactions, including the formation of a fibrous capsule consisting of inflammatory immune cells and fibroblasts around the border of the implant[45]. Although the overall inflammatory response to implanted biomaterials[45–48] must be considered, recent studies have elucidated a connection between the immune cells recruited to a biomaterial in the context of cancer and those required to establish a pre-metastatic site (Fig. 2). For example, in an immune competent Balb/C mouse, a variety of inflammatory immune cell populations were recruited to a subcutaneously implanted poly(ε-caprolactone) (PCL) micro-porous scaffold (Fig. 2a,

b). During a four-week implantation period, prior to 4T1 breast tumour cell inoculation, $Ly6C^+F4/80^-$ inflammatory monocytes and $CD11c^+F4/80^-$ dendritic cells accumulated at the implant site. However, following tumour inoculation, inflammatory monocytes further increased and $Gr1^{hi}CD11b^+Ly6C^-$ MDSCs accumulated at the scaffold (Fig. 2c), while dendritic cell and $F4/80^+CD11b^+$ macrophage populations decreased, thus recapitulating elements of the pre-metastatic niche and enabling tumour cell recruitment (Fig. 2d)[26]. In a separate study, poly-L-lactic acid (PLA) microspheres have been shown to recruit CD11b+ monocytes to the implant, which subsequently led to enhanced B1F10 melanoma cell homing at the implant site[22]. These studies indicate tumour cells can home to an implant due to the local foreign body response alone. Importantly, the composition of the immune cells in the foreign body response may differ in tumour-bearing relative to healthy animals, with the foreign body response in tumour-bearing hosts facilitating formation of a pre-metastatic niche at an ectopic location[26]. Therefore, the emerging mediatory role of the immune system for tumour cell recruitment to an implanted biomaterial has significant implications in the study of metastatic cell trafficking, as well as enabling detection and modulation of tumour cells at user-defined, ectopic locations.

2.2. Soluble factors

Chemokines and cytokines that actively influence both immune and metastatic cell behaviour play an important role in niche formation (Supplementary Table 1). For example, secreted factors from stromal cells have been implicated in recruiting immune cells associated with the pre-metastatic niche, including SDF-1, TGF-β, S100A-8/9, IL-1, and caveolin-1[49]. Similarly, immune factors including VEGF, IL-6, IL-1, TNFα, CCL17, G-CSF, Bv8, S100 proteins, CCL2 and CCL22 were shown to be overexpressed at target organs during metastatic progression, suggesting a role in pre-metastatic niche formation and tumour cell recruitment[10,43,49]. Also, VEGF was found to recruit VEGFR1+ BMDCs[4], G-CSF mobilizes MDSCs[5], Bv8 promotes angiogenesis and mobilization of myeloid cells[8], IL-6 is responsible for tumour promoting inflammation[50], CCL2 recruits monocytes and BMDCs and facilitates the extravasation of cancer cells[12] and TNFα induces S100A8/9 expression which in turn attracts Mac1+ myeloid cells and tumour cells[51,52]. Additionally, inflammatory Mac1+ monocytes and lung endothelial cells are known to secrete calcium-binding S100A8 and S100A9 factors in the presence of a primary tumour, which initiates the recruitment of additional monocytes to pre-metastatic sites[15,51]. S100A8 and S100A9 are known to increase formation and activation of invadopodia via p38 signaling, which may promote tumour cell adhesion[53]. Immature Gr1+CD11b+ MDSCs are responsible for suppressing IFN-γ and increasing inflammatory cytokine expression, and induce the expression of MMP9 in cells to allow for matrix remodeling at the niche[54].

3D scaffolds have been used to recruit metastatic melanoma tumour cells *in vivo*[22] and to characterize the role of soluble factors in mediating metastasis to bone tissue *in vitro*, where tumour cells actively prepare the site for colonization through the release of cytokines such as IL-8[55,56]. Colonizing breast tumour cells produce osteoclast-activating factors, including IL-6, IL-11, and TNFα, to initiate bone resorption and create space for a metastatic lesion[57]. Subcutaneously implanted chemokine releasing scaffolds have instead been used to compare

two factors implicated in melanoma metastasis, SDF-1 and erythropoietin (EPO), with EPO scaffolds having increased tumour cell recruitment[22] (Fig. 3a).

A related strategy used virus delivery from biomaterials that encode for chemokines to modulate immune cell trafficking[58]. Similarly, PLG scaffolds with an immobilized lentivirus encoding for CCL22 modulated the immune cell composition within the scaffold[27] resulting in an increase in MDSCs at the niche, which in turn enhanced tumour cell recruitment to the scaffold, similarly to that described in the natural niche[36,59]. These factors are thought to modulate the chemokines at the local environment; however, altering the trafficking of immune cells locally may potentially have an impact systemically. Collectively, these studies indicate that individual secreted factors have distinct cell recruitment abilities and direct release from the material may enable studies of immune and metastatic cell trafficking (Fig. 3b).

Silk biomaterial scaffolds have been developed to study the impact of BMP-2 on bone metastasis [60][60], since BMP-induced transcriptional pathways are activated during breast and prostate cancer invasion and bone metastasis[61,62]. Using a layered scaffold system, BMP-2 release stimulated the adhesion of PC3 prostate cancer cells to the scaffold and enhanced the expression of osteogenic markers. More recently, the immune cell secretome from a tumour-bearing mouse, thought to contain factors that mediate the attraction MDA-MB-231 breast cancer cells to engineered niches, was characterized with a combined approach of systems biology and biomaterial techniques[35]. Using mass spectrometry proteomics, 144 proteins were identified as uniquely secreted by the immune cells from diseased mice and were considered candidate mediators of metastatic cell homing. Using a complementary systems biology approach via measurement of large-scale transcription factor activity and subsequent computational network analysis, the list of candidate factors was narrowed to five. Haptoglobin, a secreted glycoprotein highly abundant in patients with inflammatory diseases and many types of cancer[63–67], was identified as a critical mediator of homing. This key discovery then allowed PLG scaffolds to be engineered to specifically release haptoglobin at the site of implantation in orthotopic breast cancer mouse models. These protein-releasing scaffolds recruited significantly more metastatic tumour cells to the implant, compared to blank scaffolds, indicating a role for haptoglobin in breast cancer cell homing. Taken together, elucidation of the ability of secreted factors to recruit tumour cells to engineered niches indicates that these platforms can serve to validate components of the pre-metastatic niche and also facilitate the discovery of novel contributors to pre-metastatic niche formation and function.

2.3. Exosomes

Soluble factors that elicit dramatic changes in immune cell trafficking and the target organ ECM have similarly been characterized in exosomes. Typically 30–150 nm in diameter, exosomes are small membrane vesicles shed from cells[68–71] and have been delivered locally as a means to promote tumour cell recruitment. The multi-vesicular bodies carry signalling molecules, secreted and internalized by different cell types, and participate in intracellular communication[72,73]. Exosomes were shown to prepare organs for tumour cell colonization and mobilize BMDCs to pre-metastatic niche sites[3,14]. For pre-metastatic niche formation in

the lung, RNA molecules from tumour-shed exosomes activated the innate pattern recognition receptor TLR3 in alveolar type II cells, which stimulated neutrophil recruitment to a target site[9]. As such, tumour derived exosomes incorporated in engineered pre-metastatic niches may further elucidate their role during metastatic progression[74]. Applying this concept, exosomes in a 3D biomaterial scaffold can serve as a metastatic trap (M-Trap)[25]. The M-Trap device preferentially captured metastatic cells in both peritoneal and orthotopic models of ovarian cancer (Fig. 3c). As a result, mice implanted with M-Trap scaffolds survived significantly longer than those without implants, with improved overall survival demonstrated upon removal of the implant carrying the metastatic disease. As the collective understanding of how exosomes participate in the preparation of the niche expands[13,14], biomaterials may serve as a novel tool to evaluate metastatic cell recruitment to a niche as a function of exosome presence (Fig. 3d).

2.4. Extracellular matrix

Tissue engineering strategies have been utilized to model organ-specific colonization, or organotropism, using *in vitro* mimics of the organ ECM. Tumour cell lines show a preference for the ECM according to integrin expression[75], leading to the hypothesis that integrin binding dictates organotropism, with β_1, α_2, and α_6 integrin subunit expression determining cellular adhesion to lung, liver, and brain ECM mimics[76]. By taking advantage of the cell surface receptors expressed on tumour cells, tissue-inspired biomaterials (such as bone, brain, and lung ECM) can recapitulate the integrin-mediated phenotypes and provide an "*in vitro* fingerprint" for cells with predictable metastatic targets. Further studies have determined that tumour-derived exosomes display distinct integrin patterns that preferentially bind to organ-specific cells, thus demonstrating that organotropism can be mediated through "packets" of extracellular signals[14].

Tumour cell adhesion has also been tested using decellularized matrices to coat biomaterial scaffolds[77]. Organ decellularization is a commonplace tissue engineering method used to retain the active components of the matrix[78] and has been recently used to assess tumour cell activity on primary tumour[79], lung[80], and bone-derived matrices[81]. Using this approach *in vivo*, decellularized lung and liver matrices obtained from tumour-bearing mice was used to coat micro-porous PCL scaffolds, and upon subcutaneous implantation, was shown to enhance tumour cell colonization at the scaffold (Fig. 4a, b). Interestingly, proteomics was used as a technique to evaluate the matrix composition and identify the unique components of organ-specific pre-metastatic niches[77]. In this example, myeloperoxidase, an enzyme that generates reactive oxygen species[82,83], was determined and validated as a factor that mediates tumour cell colonization using an engineered myeloperoxidase-coated PCL scaffold. Another study investigated metastatic breast cancer cell colonization using scaffolds seeded with primary human osteoblasts to prepare a mineralized bone ECM mimic[84]. The myofibrillar network produced by seeded osteoblasts, investigated using scanning electron microscopy, was found to be comparable to the assembly of trabecular bone tissue. Atomic force microscopy was also used to measure the detachment force of various breast cancer cells as a measure of tumour cell adhesion to the engineered sites. Tumour cells seeded on the human bone mimic revealed gene expression changes in osteopontin, consistent with tumour cells colonizing bone tissue *in vivo*. Taken together,

these results demonstrate the ability of combinatorial approaches to recapitulate elements of the *in vivo* niche[84], and represent a highly controllable platform to study these interactions.

2.5. Manipulation of cell populations

A variety of cell types have been implicated in pre-metastatic niche formation, yet manipulation techniques (using, for example, transgenic strategies, antibody depletion, or adoptive transfer) can affect a population systemically. Alternatively, biomaterial scaffolds can be used as *in vivo* implants to recreate defined conditions. For example, the most successful approach, where cell transplantation has facilitated cancer cell recruitment, is a bone marrow niche mimic recreated by the transplantation of human bone marrow stromal cells on silk (Fig. 4c, d)[34], BMDCs on polyacrylamide[23,85], or mesenchymal stem cells on polyurethane[86]. These cells were initially cultured on the engineered scaffold (Table 2) *in vitro* and, upon implantation, the niches were able to recruit human breast cancer cells[23,34,87], as well as erythroleukemia (Fig. 4e, f)[85], acute myeloid leukemia [86], and prostate cancer cells[23,88]. Interestingly, studies suggest the frequency of capturing tumour cells using scaffolds seeded with BMDCs may correlate with the frequency of CTCs in the blood[23]. In sum, cell-laden materials are capable of capturing tumour cells at an ectopic site using animal models of both hematological and metastatic carcinoma origin.

Aside from bone marrow mimics, tissue engineered constructs have been used to deliver stromal cells (e.g. neutrophils, fibroblasts, lymphatic endothelial cells, or osteoblasts) at target organs where they provide a permissive microenvironment for human breast cancer cell colonization[89,90]. Local fibroblasts that participate in the formation of pre-metastatic niches become cancer-supportive through the secretion of growth factors and ECM remodeling proteins[91]. In a model of ovarian and colorectal peritoneal metastasis, cancer-associated fibroblasts (CAFs) were encapsulated within alginate/gelatin microparticles (500–700 μm in diameter), coated with a membrane composed of polyelectrolytes to retain the CAFs and prevent degradation. Once implanted in the intraperitoneal space of nude mice, CAFs and CAF-secreted ECM were found to be key in the formation of peritoneal niches for metastasis[92]. Injection of MP-CAFs into the peritoneal cavity redirected cancer cells to the microparticles and resulted in a biomimetic trap that prolonged animal survival[93]. Similarly, MDSCs have been harvested from spleens of mice and seeded onto PLG scaffolds prior to implantation in an orthotopic model of breast cancer[27] using highly metastatic, brain-tropic MDA-MB-231BR cells[94]. MDSCs were retained on the scaffold after implantation, and recruited significantly more tumour cells to the implant site relative to blank scaffolds. Recent protocols have also been developed to engineer humanized bone tissue using electrospun PCL-tricalcium phosphate scaffolds seeded with human osteoblastic cells to mimic clinical bone metastases[95]. Using the bone mimic within humanized mouse models, the study modelled several stages of the human bone metastatic cascade, including spontaneous metastasis from orthotopic prostate tumours, systemic metastasis, and local bone colonization.

3. Tumour cell behaviour at engineered niches

Once a DTC adheres to and grows within a niche in the target organ, the cell is said to have colonized the organ. Colonization has been associated with specific genetic changes, including a mesenchymal-to-epithelial (MET) transition. In contrast to the EMT transition during invasion, MET is the process by which tumour cells return to their epithelial-like state to form a distant tumour mass. MET is typically characterized by gene expression studies, generally showing a return to E-cadherin expression and down-regulation of vimentin[96]. Metastatic colonization is also mediated by the activity of specific transcription factors, including decreased transforming growth factor β/mothers against decapentaplegic homolog 3 (TGFβ/SMAD3) canonical signalling activity, and the loss of the paired related homeobox factor (PRRX1) activity, both potent EMT inducers[97,98]. As DTCs successfully colonize the target organ, proliferation at the metastatic site may occur based on cues received from the pre-metastatic niche[2]. The cues involved are still largely unknown; however, there is evidence that the perivascular niche, as well as sprouting and stable endothelial networks, regulate dormancy through control of thrombospondin 1 (TSP-1), TGF-β, periostin, tenascin, versican, and fibronectin, all factors previously implicated in the pre-metastatic niche[99]. Without proper activation, tumour cells may undergo apoptosis at the target organ, remain dormant at the metastatic site for up to several years, or continue circulating through the body[100–102103].

The inability of DTCs to grow at a metastatic site[104–106], a part of metastatic inefficiency, has been modelled using *in vitro* colonization experiments where the presence of specific ECM proteins can activate dormant CTCs back into a proliferative state. Using a 3D basement membrane culture system, solitary tumour cells can remain dormant due to cell cycle arrest through elevated abundance of cyclin dependent kinase inhibitor proteins p16 and p27[24,107]. In addition, the proliferation rates of a variety of breast cancer cell lines, measured using 2D and 3D basement membrane gels, pointed to signs of dormancy in the 3D culture *in vitro*. However, the introduction of fibronectin to the 3D culture environment enhanced proliferation rates of dormant cells and increased cytoskeletal rearrangements, consistent with a static to dynamic switch in phenotype.

The effect of tissue paracrine signalling on metastatic cells, as determined using 3D co-culture systems, can also be used to refine pre-metastatic niche models[108]. For instance, to recreate MDA-MB-231 tumour cell extravasation, the bone pre-metastatic niche was recently reproduced in 3D using a microfluidic platform consisting of osteo-differentiated mesenchymal stem cells embedded in a collagen gel lined with endothelial cells[109]. Likewise, 3D collagen gels containing human lung adenocarcinoma cells, lung fibroblasts, and macrophages were used to track matrix metalloproteinase 1 (MMP-1) and VEGF production in different culture conditions (e.g. hypoxia)[110]. Co-culture systems on a silk scaffold of human breast adenocarcinoma cells with osteoblast-like cells and mesenchymal stem cells have also resulted in enhanced migration, adhesion and drug resistance[111]. When compared to the same cells co-cultured in 2D on standard tissue culture plastic, the study further reported phenotypic changes in the niche osteoblasts, including decreased proliferation and mineralization, concomitantly to enhanced tumour cell activity[111]. A similar study was performed where the LNCaP cells were embedded in poly(ethylene

glycol) (PEG) hydrogels and cultured with PCL scaffolds pre-seeded with human osteoblasts[112]. Following microarray analysis of cells obtained from two engineered scaffolds, the study revealed that paracrine signalling between cancer cells and osteoblasts altered the expression patterns of genes associated with homing and colonization (such as S100A6), compared to mono-culture controls.

4. Translational opportunities for pre-metastatic niche mimics

Implantable niches may serve as *oncomaterials,* a term we propose and define as biomaterials for oncology that enable the detection and the treatment of cancer metastasis (Fig. 5a, b). In a clinical setting, the probability of a tumour spreading to target organs has been shown to correlate with tumour size; for example, breast cancer tumours less than 1 cm in diameter have a lower risk of metastasis[113]. Detection strategies to map metastatic spread mostly rely on whole body imaging modalities such as Positron Emission Tomography (PET), x-ray Computed Tomography (x-ray CT) and Magnetic Resonance Imaging (MRI); however, the initial cell clusters are beyond the resolution of these imaging systems. These limitations are particularly problematic for highly aggressive cancers that follow a parallel progression model, where tumour cell dissemination and colonization occurs during the undetectable stages of the primary disease[114].

4.1: Materials for metastatic cell detection

The early detection of rare CTCs in the blood may enable earlier treatments for metastatic cancer[115], which has motivated the continued development of nanomaterials to isolate and characterize CTCs[116]. To date, genetic screening of tumour biopsy samples has been the most common approach for identifying biomarkers to developing personalized therapies. However, the progression of a cancer from a neoplastic or dysplastic lesion to metastasis is increasingly understood as the result of continued evolutionary pressure that dictates mutations of its genetic and molecular landscape. This dynamic behaviour is the primary factor responsible for the emergence of therapeutic-resistant clones and challenges the development of personalized therapies. For these reasons, techniques to capture, characterize, and culture CTCs are intended to complement primary tumour biopsy analysis and provide a comprehensive disease description for individual patients[31,116,117]. CellSearch for example, an FDA approved, commercially available CTC enrichment system, enables reliable detection of CTCs in blood samples from metastatic cancer patients[118]. Most notably, *ex vivo* culture of CTCs in conjunction with *in vitro* biomaterial mimics of the pre-metastatic niche have facilitated the capture, culture, and study of CTCs[119–121].

CTCs are isolated from blood, whereas cells found within the pre-metastatic niche mimics have left the vasculature and may represent a distinct cell population with distinct prognostic value. Despite advances in *ex vivo* detection, CTCs may remain in the circulation for years, and those captured in blood samples may therefore not be representative of tumour cell populations capable of homing and colonization[31,101,122], because the detection of CTCs does not indicate the existence of permissive niches. Recently, biomaterial scaffolds for the early detection of cancer metastasis[27] were reported in an orthotopic mouse model of breast cancer. Micro-porous PLG scaffolds were implanted, either subcutaneously or in the

intraperitoneal fat, and tumour cells populated these scaffolds prior to their colonization at common organ sites (i.e. lung, liver, and brain). Interestingly, using inverse spectroscopic optical coherence tomography (ISOCT)[123], unique microstructural alterations were detected at the scaffold due to tumour cell arrival, allowing for a non-invasive and label-free detection method of metastatic colonization. This type of scaffold technology, coupled with ISOCT or other imaging techniques, may enable a viable method for early detection during low metastatic tumour burden (Fig. 5c). In a translational setting, these scaffolds alone, or modified with ECM proteins or cytokine delivery, could provide direct access to actively colonizing tumour cells for patient-specific phenotypic and genomic analyses.

4.2: Early intervention for metastatic cell capture can enhance survival

Implantable scaffolds have been shown to significantly increase survival in mouse models of metastasis. For instance, micro-porous PCL scaffolds have increased survival of immune competent mice inoculated with 4T1 metastatic breast cancer cells[26]. This scaffold provided a site for early detection and acted as a “sink” for metastatic tumour cells (Fig. 2d) and myeloid derived suppressor cells (Fig. 2e). As a result, the scaffold reduced the average tumour burden in the liver and brain (Fig. 2f, g). A post-surgical model of breast cancer metastasis was then used to investigate the impact of the scaffold on survival where the primary tumour was removed following the localization of tumour cells in the scaffolds. 40% of scaffold-implanted mice survived the tumour resection procedure past 200 days relative to sham controls where survival did not exceed 30 days (Fig. 2h). The study suggested that increased survival may result from a decreased burden of MDSCs present at the primary tumour and spleen of scaffold-implanted mice (Fig. 2i). Therefore, the study implicates that biomaterials designed to reduce the overall generation of MDSCs during metastatic disease progression or divert them to an ectopic location may impact survival. Similarly, exosome impregnated scaffolds drastically changed the pattern of peritoneal ovarian cancer metastasis by redirecting the vast majority of tumour cells to the implant (Fig. 3c)[25], which resulted in a significant survival benefit for mice that received an implant (mean survival of ~200 days compared to ~120 days). Additionally, removal of the implant after focalization of the disease to the biomaterial further enhanced survival (~310 days mean survival).

4.3: Opportunities for metastasis detection platforms

Although recent evidence suggests that pre-metastatic niche models enable the early detection and treatment of metastatic disease[27], open questions remain regarding the efficacy of these platforms when compared to other emerging metastasis detection technologies. Additional technologies for metastasis detection include exosome detection and CTC enumeration (Table 3). Both platforms are part of a larger initiative to utilize liquid biopsies to gain more information about a patient’s disease state, evolving molecular features, and response to therapy[118,124]. Advantages to liquid biopsy strategies for detection include the ease of sample collection and ability to collect multiple samples over the course of a patient’s treatment. While liquid biopsies have shown promise in these areas, they also have distinct disadvantages that could potentially be circumvented by pre-metastatic niche mimics. For example, exosome detection is likely to be less sensitive than CTC detection due to exosome heterogeneity and their presence in large numbers also in healthy

patients[68,125]. Similarly, the presence of CTCs indicates the risk for metastasis but does not indicate the presence of permissive microenvironments in organs for these cells to home and colonize. These considerations show specific advantages for the use of pre-metastatic niche mimics (Table 3), however, there are also potential issues associated with the clinical use of these devices, such as the overall safety of creating a site for metastatic cells to home that will need to be evaluated thoroughly in clinical trials. Future methods may provide complementary implementations of these techniques to provide a more comprehensive evaluation on the metastatic state of a patient.

In a clinical setting, the choice of material (Table 2) is critical for designing a functional implantable device for recruiting and detecting metastatic cells. For example, materials such as PLG are susceptible to hydrolytic degradation, thus limiting the amount of time the material can remain in a patient. The scaffold should ideally maintain its structure for several months during a patient's treatment, given that metastasis may occur on a timescale from months to years[126]. Polymer scaffold degradation is usually desired for tissue engineering applications where host cells eventually replace the material, but degradation is likely undesirable for long-term implantable metastatic detectors. Non- or semi-degradable materials could be used to fabricate implantable scaffolds less susceptible to hydrolytic degradation[32]. The material should also elicit an appropriate inflammatory response at the implant site to initiate the recruitment of metastatic cells, and should be amenable to harvesting intact populations of tumour cells for downstream analysis[77]. Additionally, scaffold porosity increases the interior surface area for blood vessel and immune cell infiltration to provide tumour cells access to the scaffold[32]. Material selection is paramount for successful translation of pre-metastatic niche mimics as oncomaterials.

Thus far, no clinical trials have been initiated for the application of biomaterial pre-metastatic niche mimics. Although several biomaterials utilized as pre-metastatic niche mimics are already FDA approved for use in human patients (Table 2), limitations in imaging tumour cell arrival at the implant remain. ISOCT is a practical approach for detecting the nanostructural alterations due to tumour cell arrival[123], however, the penetration depth that is associated with this optical technique will need to be enhanced for translation to a clinical setting. Imaging technologies, such as ultrasound[127], are already available in the clinic and may be implemented for tumour cell detection at a scaffold. Even though safety remains an open question, the future for pre-metastatic niche mimics as oncomaterials remains promising.

5. Opportunities and Conclusions

Pre-metastatic niche mimics offer the ability to identify and validate critical factors leading to metastatic cell colonization at an ectopic site. Roles of inflammatory immune cells, secreted factors, exosomes, ECM proteins, and delivered cells have been evaluated using niche mimics to determine contributions to metastatic cell homing and colonization. Furthermore, the capture of early metastatic cells at a pre-defined site may enable early detection of metastatic cell dissemination. The development of novel imaging modalities, or the engineering of probes to label colonizing tumour cells may enable real-time tracking of tumour cells or vascular leakiness at the niche during the evolution of the disease. Capturing

tumour cells at an ectopic site could potentially reduce the burden of disease in solid organs and provide an extended window of time over which a therapeutic intervention may succeed. The use of oncomaterials supplemented with current therapeutic strategies such as surgery and chemotherapy may serve as a disruptive technique for combating metastasis. Extending beyond the concept of capturing tumour cells, scaffolds may be bioengineered to manipulate other types of circulating niche components, including exosomes and immune cells that reflect disease (e.g., MDSCs). Furthermore, future work in the genetic profiling of captured metastatic cells at implanted niches may lead to the identification of the types of cells arriving at the scaffold (e.g. tumour stem cells, EpCAM+ cells), which may in turn guide the discovery of targets to treat metastasis based on the disease biology. In conclusion, the successful integration of pre-metastatic niche components in biomaterials can enable the discovery of biomarkers and other molecular cues leading to metastasis, and could be developed further as diagnostic and therapeutic platforms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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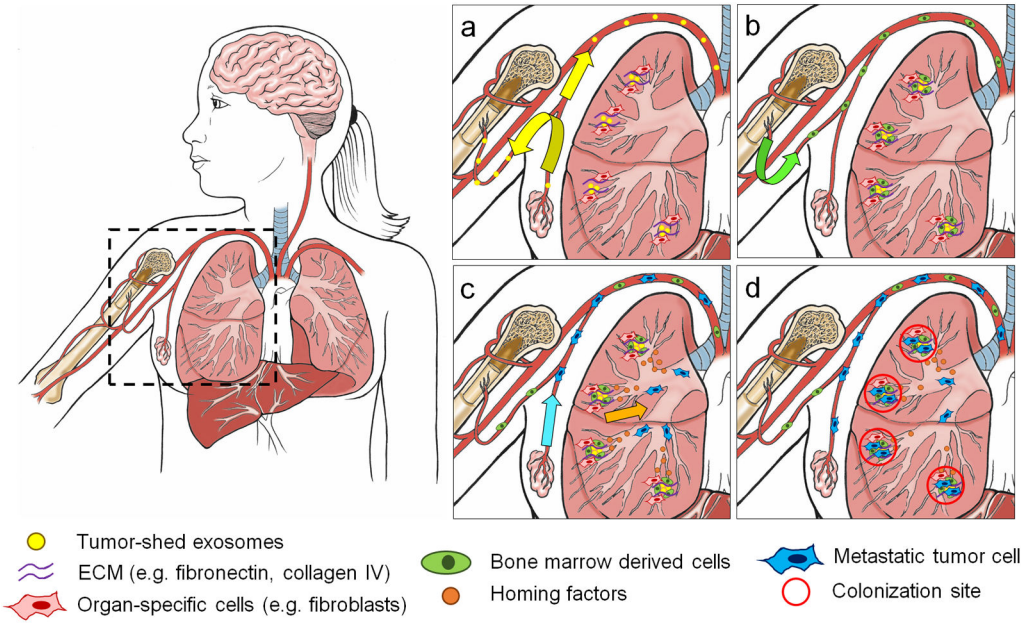

Figure 1.

Formation of the pre-metastatic niche. (a) Hypoxic tumour sheds exosomes (yellow dots) to simultaneously prepare the niche at a target organ by fusing to organ-specific cells (red cells, e.g. fibroblasts) and to stimulate mobilization of BMDCs. Other tumour-secreted factors (e.g. lysyl oxidase) crosslink ECM proteins (purple curves). (b) BMDCs (green cells) accumulate at conditioned sites, adhering to accumulated ECM. (c) BMDCs and other immune cells (e.g. myeloid derived suppressor cells) secrete factors (orange dots) to induce metastatic cell (blue cells) homing to niche sites. (d) Metastatic cells colonize and proliferate at metastatic niche sites. Core illustrations courtesy of Katie Aguado.

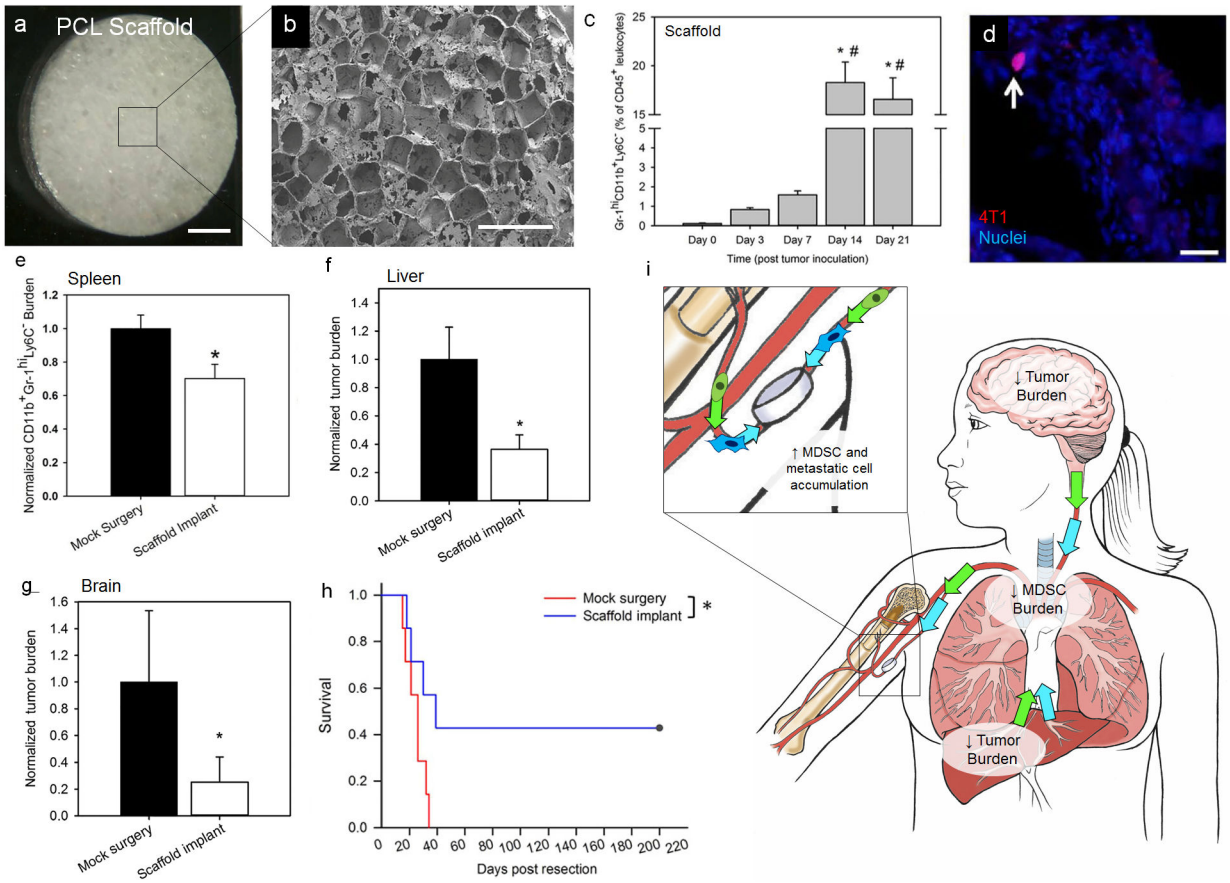

Figure 2.

Myeloid derived suppressor cell (MDSC) and metastatic cell trafficking in a breast tumour bearing mouse implanted with a biomaterial scaffold. (a) Photographic (scale = 1 mm) and (b) scanning electron microscope images (scale = 1 mm) of a microporous PCL scaffold. (c) Tumour progression influences $Gr1^{hi}CD11b^{+}Ly6C^{-}$ MDSCs accumulation at the PCL scaffold implanted subcutaneously in a Balb/C mouse inoculated with 4T1 triple negative breast tumour cells. (d) White arrow indicates tdTomato+ 4T1 cell among a cluster of cells localized to the scaffold. (e) PCL scaffolds reduce MDSC burden in the spleen, which indicates a reduction in systemic MDSC burden. Reduced tumour burden in mice receiving a scaffold implant is observed in the (f) liver and (g) brain (*$P < 0.05$). (h) Improved survival for tumour-resected mice receiving a scaffold implant relative to mice undergoing a mock surgery (n=7 for each group, *$P < 0.05$). (i) Proposed mechanism for MDSC and metastatic cell trafficking after scaffold implantation and tumour resection, with reduced MDSC burden in the circulation and reduced tumour burden in the liver and brain, with subsequent increased MDSC and metastatic cell accumulation at the scaffold. Figures reproduced with permission from the American Association for Cancer Research[26]. Core illustrations courtesy of Katie Aguado.

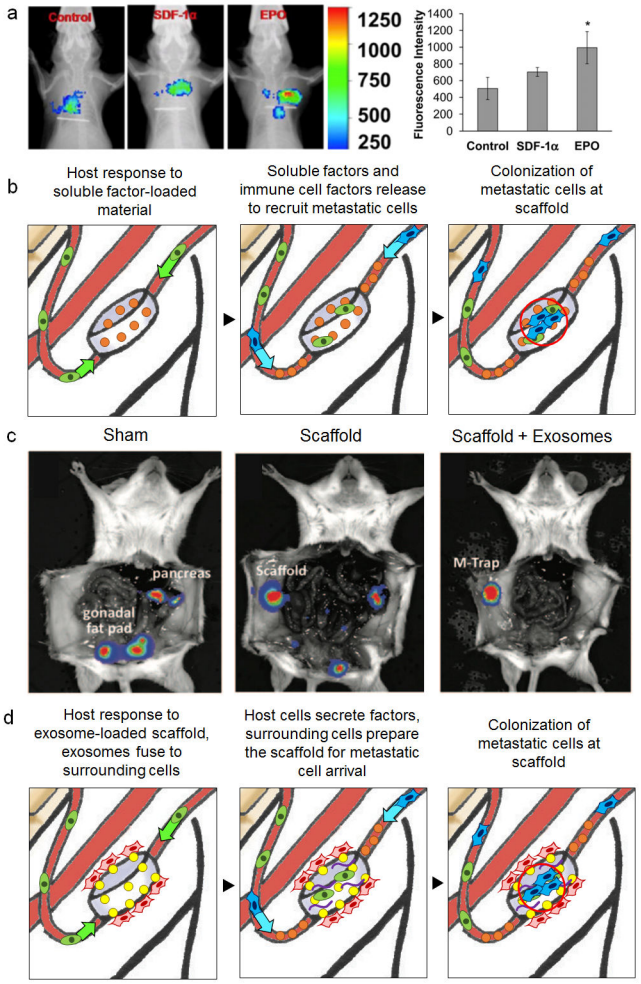

Figure 3.

Biomaterials loaded with soluble factors and exosomes mediate tumour cell homing. (a) Control, EPO and SDF-1α loaded scaffolds recruit labeled B16F10 melanoma cells, quantified using bioluminescence imaging (*$P < 0.05$). Figures reproduced with permission from Elsevier[22]. (b) Proposed mechanism for biomaterials pre-loaded with soluble factors of interest in mediating the recruitment of metastatic cells. (c) Exosome-laden scaffolds (M-trap) capture SKOV3 ovarian cancer cells delivered into the peritoneal cavity. Bioluminescence imaging shows control mice with metastasis to the pancreas and gonadal fat pads 1 week after inoculation. Blank scaffolds redirected tumour cells to the implant site, although abdominal metastases were still detected. M-trap scaffolds were able to recruit tumour cells with no visible metastases at 1 week after inoculation. (d) Proposed mechanism for exosomes in mediating preparation of the pre-metastatic niche at the scaffold. Figures reproduced with permission from Oxford University Press[25].

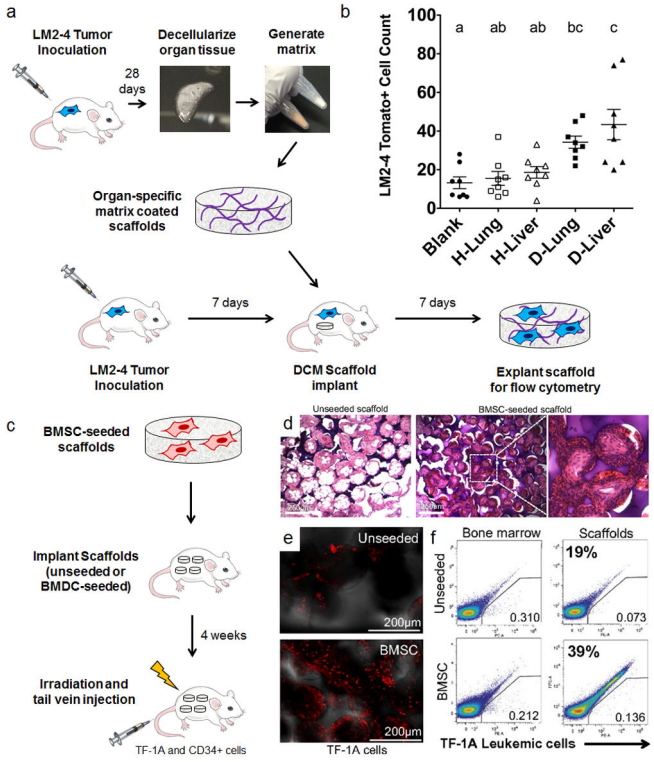

Figure 4.

Modelling organotropism using ECM- or BMSC-functionalized scaffolds. (a) Decellularized lung and liver matrix from healthy and diseased mice inoculated with tdTomato-tagged LM-2 lung/liver targeting breast tumour cells was used to coat PCL scaffolds, and scaffolds were implanted subcutaneously in tumour-inoculated mice to detect differences in tumour cell colonization as a function of matrix coatings. Mouse image drawn by Katie Aguado and reproduced with permission from Nature Publishing Group[35]. (b) Matrix-coated scaffolds from diseased lungs and livers recruited more cells relative to blank and healthy coating controls as assessed by flow cytometry. Groups with different letters are significantly different ($P < 0.05$). Figures reproduced with permission from Elsevier[77]. (c) Delivery of multipotent BMSCs ($CD44^+$, $CD106^+$, $CD14^-$, $CD34^-$, $CD45^-$, $CD73^+$, and $CD105^+$) on scaffolds recruit TF-1A leukemia cells to an implant site. (d) Images of H&E stained tissue sections of subcutaneously implanted 3D microfabricated polyacrylamide scaffolds (unseeded vs. BMSC seeded, scale bars = 250 μm). (e) Homing of intravenously transplanted human TF-1A cells to unseeded vs. BMSC seeded scaffolds. Confocal images of scaffolds show significantly more stained TF-1A cells arriving to BMSC-seeded scaffolds 6 hours after injection (scale bars = 250 μm). (f) Flow cytometric analysis of labeled TF-1A cells at the bone marrow vs. implanted scaffolds. FACS analysis suggests there were approximately twice as many cells at BMSC-seeded scaffolds relative to unseeded scaffolds. Figures reproduced freely under open access from the National Academy of Sciences[85].

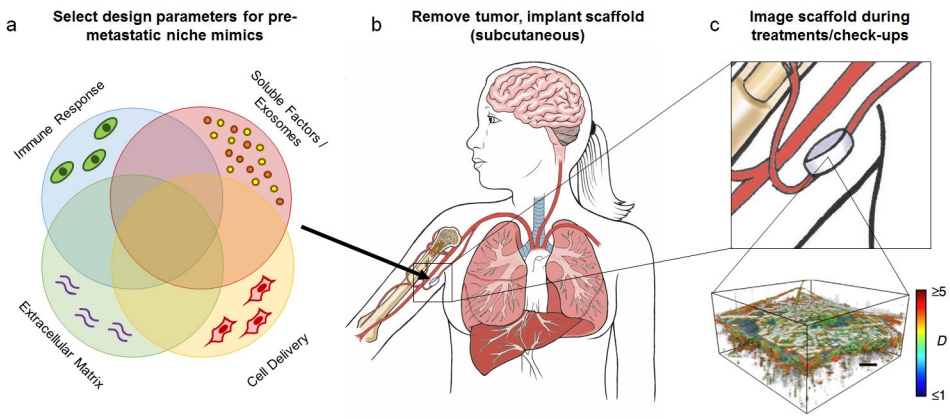

Figure 5.

Proposed detection strategy for metastatic breast cancer. (a) Pre-metastatic niche oncomaterials may be designed from a variety of parameters, including the natural immune response to the implant, soluble factor delivery, extracellular matrix, and cell delivery. Parameters may be tuned depending on the cancer or the needs for a specific patient for designing the most effective oncomaterial. (b) After removal of the primary tumour, a biomaterial scaffold may be implanted subcutaneously, ideally before metastasis occurs. (c) Regular imaging at check-ups may be performed during the patient's course of treatment. When using ISOCT, the shape factor (*D*) may be used to quantify microstructural alterations at the scaffold due to the arrival of metastatic tumour cells (scale bar = 200 μm). ISOCT image reproduced with permission from Nature Publishing Group[27]. Core illustrations courtesy of Katie Aguado.

Table 1

Strategies for characterizing the pre-metastatic niche and metastasis formation

Strategy	Advantages	Disadvantages
Biomaterial pre-metastatic niche mimic	• Limits off-target effects • Defined location for analysis • Biomaterial properties can be manipulated for different applications • Ease of evaluating multiple niche cues in one device • Large number of cells can be retrieved from the device	• Does not recapitulate all elements of native pre-metastatic niche • Foreign body response may influence the biomaterial environment and differ from a natural pre-metastatic niche
High risk tissue bed biopsy	• Enables determination of cues leading to organ-specific metastasis • Captures heterogeneity between metastatic foci	• Variability between samples may confound discovery of critical signals • Identification pre-metastatic sites is limited
Tumour cell modification	• Direct evidence for molecular drivers of metastasis • User-defined alterations	• Potential for off target effects on tumour progression • Generating a reliable cell/mouse model is challenging
Genetically engineered mouse models (GEMM)	• Direct evidence for role of a factor or cell type in metastasis • Ability to knock-out and knock-in specific genes	• Costly and time-intensive • Potential for off-target effects on health of the animal or tumour progression

Table 2

Materials and biological modifications to engineer the pre-metastatic niche

Source	Material	Structure	Fabrication Method	Bioactive Modifications	Reference(s)
Synthetic	Poly(lactic-co-glycolic acid)	Scaffold	Gas foaming	CCL22, MDSCs	27
	Poly(lactic-co-glycolic acid)	Layered scaffold	Microspheres pressed in gas foamed scaffold	Haptoglobin	35
	Poly(ε-caprolactone)	Scaffold	Gas foaming	None	26
	Poly(ε-caprolactone)	Scaffold	Gas foaming	Exosomes	25
	Poly(ε-caprolactone)	Scaffold	Electrospinning	Osteoblasts	33
	Poly-L-lactic acid	Microparticles	Precipitation	EPO, SDF-1	22
	Hydroxyapatite	Nanoparticles in PLG scaffold	Precipitation, gas foaming	Serum protein	56
	Polyacrylamide	Porous gel	Microfabrication	Collagen I, BMSCs	23,85
	Polyurethane	Scaffold	Commercially available	MSCs	86
	Polyallyamine/polystyrene	Microparticles	Layer-by-layer coating	CAFs	93
Natural	Bone fragments	Human/mouse sources	Direct harvest	None	128,129
	Silk	Scaffold	Salt leaching	BMP-2	34,87,130
	Lung/liver matrix	Coatings	Decellularization	None	77
	Osteoblast matrix	Mineralized sheets	Decellularization	None	84
	Collagen	Bulk gel	Embedded in microfluidic chamber	Osteo-differentiated MSCs, Endothelial cells	109

Table 3

Risks and opportunities of detection platforms for metastasis

Detection Platform	Stage at detection	Safety	Sensitivity	Specificity	Therapeutic benefit	Reference(s)
Exosome detection	Primary	+	–	+	–	124
CTC detection	Circulation	+	+	+	–	118,131
Biomaterial pre-metastatic niche mimic	Dissemination	?	+	++	+	23,25–27