Biomaterials and regenerative medicine in the treatment of cancer

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Cancer continues to be a field of medical research and practice demanding complex and expensive forms of therapy, many of which still involve the systemic administration of chemotherapeutic agents. This latter form of treatment carries with it many complications and unwanted side-effects. Unfortunately, up until now relatively few research groups in the biomaterials field have focussed their attention on oncology, with the result that a number of innovative developments, which could benefit cancer patients, have remained in the realm of non-oncological regenerative medicine. Of special interest for oncology is the progress which has been made in the development of interactive and responsive biomaterials, as these can be tuned to release signal molecules or therapeutic agents in response to a local microenvironment. This strategy has the double advantage of local release of anti-cancer agents and at the same time modulation of the healing response in the affected tissues. To achieve such functionality many advances, especially in polymer chemistry, but also in ceramics and metals, have led to the development of suitable targeted biomaterials. This has been greatly assisted by innovations in nanotechnology, including the versatility of core-shell nanoparticles for nanomedicine. For the latter a number of *in vitro* models, especially of barrier systems in the human body have been devised as models for targeted therapy. Moreover, modern biodegradable, responsive hydrogels could be used along with complex models for tumour biology, such as three-dimensional (3D) spheroid culture systems, which can be applied to investigate, for example, the processes of invasion and the role of inflammation in cancer. The necessity for suitable models to address biomechanical aspects represents a further issue of importance in oncological research and combines the expertise of both physics and biology. Finally, the complex field of mechanobiology can only be successfully addressed by interdisciplinary research activity.

Keywords: cancer, biomaterials, regenerative medicine, 3D-models, nanomedicine

1. Introduction

Progress both in the pathobiology of cancer and in the fields of polymer chemistry and nanotechnology have made biomaterials highly relevant for the field of cancer treatment. The following presentation will address aspects of our current understanding of cancer and how modern approaches to biomaterials in Regenerative Medicine (RegMed) can be mirrored in relevant *in vitro* models. Adjuvant cancer treatments tend to be systemic and thus affect all regenerative processes, including those which take place in normal tissues. The corollary of this is that adjuvant therapy should, if possible, be in the form of local delivery. The latter has two main aims. First, there should be targeted destruction of cancer cells, the principal focus being cancer stem cells (CSCs), as they have the potential to give recurrent tumour and metastases. However, modulation of the tumour microenvironment is equally important, as this multifacetted entity exerts considerable influence on tumour development and progression (see Sect. 3). Among the essential elements are the type and degree of inflammation, levels of hypoxia, extracellular matrix (ECM) with its cancer-associated fibroblasts (CAFs), and angiogenesis, to mention but four of the relevant components.

2. Relevant biomaterial developments

The development of interactive and responsive biomaterials represents a major step forward in trying to control the tissue response to a biomaterial. One of the pioneers of this technology, Jeffrey Hubbell, succeeded in incorporating a biologically active moiety into a polymer network in such a way that its release would be triggered only by a cellular enzyme, that is, at the time point when relevant cells would have invaded the biomaterial [1, 2]. Other research groups have used different phenomena to fine-tune the release of signal molecules. Thus, the group of Teruo Okano in Japan synthesized specialized temperature-sensitive polyacrylamide polymers, making use of temperature-dependent molecular conformational change from hydrophobic to hydrophilic states and *vice versa*. This was

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performed in such a way that, for example, the desired biological signal molecule would be released at body temperature, but not at room temperature [3, 4]. In another approach, the Del Campo group in Germany has focused on so-called "caged molecules", which can involve release of a chemotherapeutic agent held in a light-sensitive molecule or even cells in a polymer network [5]. When light of the chosen wavelength is directed to the responsive molecule, a conformational change takes place which leads to release of the caged component.

Especially following surgical removal of malignancies there can be a large volume of excised tissue requiring regeneration, this bringing the problem of not only providing the specific tissue type required, but also an adequate vascularization to nourish the tissue [6–9]. The past decade has witnessed a great interest in decellularized matrices, as these contain topographical and molecular cues which can initiate and guide the behaviour of cellular elements which are in the immediate microenvironment of the implanted matrix, including vascular cells needed to re-establish an adequate blood flow. In the latter the Walles group has used a decellularized porcine small intestinal segment, the extensive vascular network of which can be re-seeded with endothelial cells in a true tissue engineering application [10]. Stephen Badylak in Pittsburgh has underlined the importance of the matrix pre-treatment of biologic scaffolds in determining the biological reaction after implantation. Thus, chemical cross-linking, for example, elicits a pro-inflammatory macrophage phenotype (M1) in the immune response to such biomaterials [11, 12].

Many of the innovative biomaterials with responsive characteristics are polymers, which lack the necessary strength required in some areas of the body, e.g. in skin and long bones. This can be assisted by combining the softer component with a more stress-resistant material in the form of a composite [13]. In a further advance, rapid prototyping, which has been used for a long time in anatomical modeling [14], offers the possibility of 3D printing of cell constructs inside a suitable polymer matrix, this being an important technology for tissue engineering (TE) applications, especially if the printing is performed under mild conditions, compatible with biological material, including biological signal molecules and cells [15, 16]. Using computer tomographic or magnetic resonance tomographic data it is possible with this combined technology of "additive manufacturing" [17] to make patient-specific scaffolds which fit the corresponding defect perfectly. One specific example is the use of this technique for bone regeneration [18].

3. Fields of interest in biomaterials and cancer

3.1. Developing suitable targeted biomaterials

Modern polymer chemistry, with the ability to use molecular self-assembly and incorporate biological signal molecules into polymer networks to finally form hydrogels, has provided a tunable injectable system [19] which could be used as a biomaterial coating or injected into a specific anatomical location. Similarly, nanotechnology has provided a platform to synthesize sophisticated nanoparticles (NPs) which could be of core-shell nature, thus offering the possibility to modify the surface using physicochemical methods, but also fill the core with a drug delivery system [20]. Such NPs could be injected, inhaled or form part of a coating system, possibly within a hydrogel coating on, for example, an implant. Polymeric micelles containing anticancer medication are also far advanced with respect to their clinical application [21].

3.2. Developing models for targeted therapy

For clinical translation of nanoparticle (NP) delivery systems one needs to develop models of those barriers encountered by the NPs as they are applied to the human body. It is evident that such testing schemes will involve both *in vitro* and *in vivo* studies, but this report will address only the former. One of the hallmarks of barriers *in vivo* is that they are formed and functionally maintained by more than one cell type. Thus, any models *in vitro* must reflect this cellular heterogeneity, this being achieved experimentally by culturing the required cell types on both sides of a filter membrane in a so-called Transwell® system [22]. The close proximity of the cells permits cellular crosstalk in a paracrine fashion, and using such co-cultures we have been able to simulate in the laboratory the bronchial barrier [23], the air-blood or alveolo-capillary barrier in the lower respiratory tract [22], the gastrointestinal barrier [24], the skin barrier [25], and finally the most complex of all, the blood-brain barrier (BBB) [26], as the latter is composed of and maintained by three different cell types, the brain capillary endothelial cells, the pericyte and the astrocyte.

Most of these systems have been established under static conditions, but even a cursory look, for example, at pulmonary physiology makes it evident that there are mechanical forces which must be incorporated into the models. Thus, the tensional forces exerted on the respiratory barriers during inspiration and expiration cannot be regarded as trivial, but rather as a likely modulating factor in NP uptake and transport. There are also other issues which need to be reflected in the model systems, including the action of surfactant present in the alveoli [27], but also the presence of further highly active cell types, such as the alveolar macrophage [28]. Thus, coculture models represent an important instrument in adding complexity to the *in vitro* system to simulate more closely the heterotypic cell interactions *in vivo*.

3.3. Developing complex models for tumour biology

Tumour biology is multifacetted, which makes its simulation *in vitro* a challenging task. In the past years oncological researchers have realized that in studying tumour pathobiology it is insufficient to study only the malignant cells,

but also to understand the so-called "tumour microenvironment", which has a major role to play in tumor progression, but also in the efficacy of therapies [29]. This is a complex entity and consists of the tumor vasculature, both blood and lymphatic vessels, the extracellular matrix (ECM) and the contained stromal cells [30].

3.3.1. The importance of three-dimensionality (3D)

One natural approach to modeling is to look at the morphology of different types of cancer, as this permits a tissue level view rather than one at single cell level. One component of malignant tumour progression is the formation of metastases, which in many solid tumours involves lymphatic or haematogenous spread. In histological sections of cancerous tissues the pathologist often observes tumour deposits inside the lumen of such vessels. It is significant that these are not single cells but groups of tumour cells (TC), single cells entering the vascular systems being easily destroyed by the immune system. This histological observation forms the basis of the first important criterion for any relevant *in vitro* model of cancer, namely that it should be 3D. Simulation of this in a culture dish can be achieved through the cultivation of so-called multicellular tumour spheroids (MCTS), which can be made, for example, by the agarose overlay technique from many cancer cell lines [31], including those of common cancers like colorectal and breast cancer [32], but also by melanoma [33]. This very useful technique was principally initiated by Sutherland et al. [34] using a suspension culture technique for Chinese hamster V79 lung cells. These 3D spheroids had a clear zonal demarcation as seen by microscopy, that is, a puter zone of cell proliferation, an intermediate zone of stable structure and an inner zone of necrosis [34]. Since then this spheroid model has become one of the standards in cancer research [35].

3.3.2. The importance of invasion

In vitro models of cancer should also permit study of invasion. This includes all steps from epithelial-mesenchymal transformation (EMT) [36] through local extracellular matrix (ECM) invasion to intra- and extravasation of both lymphatic as well as blood vessels [37]. In studying extravasation, i.e. the invasion of TC from the lumen of a blood vessel through the wall to the extravascular space, we used monolayers of endothelial cells (EC) on top of which were placed melanoma MCTS. These studies showed that the melanoma TC use the production of reactive oxygen species to locally destroy EC and thus allow easy migration of TC out of the vessel [38].

3.3.3. The importance of inflammation

Models for cancer should also include inflammation, as this is an important element in the tumour microenvironment. In an early study of macrophage phenotypes in colorectal cancer we observed that proinflammatory macrophages (M1) are predominantly present at the invasion front, whereas uncommitted (or naive, M0) and regulatory macrophages (M2) were mostly in the non-invasive regions of the tumours [39]. We postulated that the M1 at the invasive front could reflect a promoting factor for invasion, for example, by production of proteolytic enzymes which breakdown the ECM. To test this hypothesis we added the different macrophage subtypes to colorectal MCTS on top of Type I collagen and studied TC migration out of the spheroid [32]. This study confirmed that TC migration was greatest under M1 influence.

3.4. Biomechanical aspects of tumour biology

Cross-disciplinary interactions at the interface between physics and oncology have provided new knowledge on the mechanobiology of malignancy. Thus, issues such as matrix stiffness and interstitial tissue pressure are being addressed and have already given novel insights into tumour progression. A good example is how ECM stiffness is sensed by and responded to by TC [40]. This "outside-in signaling" process involves clustering of ß1-integrins on the TC plasma membrane, with subsequent effects on the assembly of the intracellular actin-myosin cytoskeleton. This is known to involve molecules like FAK (focal adhesion kinase) and β-catenin, the latter being translocated to the nucleus, where gene expression is triggered. More recent studies indicate that the transcription activator molecules YAP-1 and TAZ are increasingly moved from the cytoplasm to the nucleus, as the ECM stiffness is increased [40]. Returning to the 3D tumour spheroids described before, Koike et al. [41] showed that different degrees of solid stress generated in agarose gels of differing mechanical properties affect the formation of spheroids. It is hoped that elucidation of the mechanosensing pathways participating in cancer phenomena such as tumour progression and therapy resistance could form the basis of new therapeutic approaches by developing inhibitors against the principal molecular players, such as integrins and their downstream signals [42].

Tissue culture models in most fields of biomedical research are dominated by static culture systems, as these are more easily generated and monitored than dynamic systems. The advent of microfluidic systems offers the possibility not only to incorporate biomechanical factors in the assays, but also to move towards miniaturization [43]. The latter presents the advantage of permitting high-throughput studies, in which cell co-cultures can be spatially controlled, signaling gradients can be generated, and perfusion can be integrated [44, 45]. It is evident that this is useful for studying factors controlling, for example, differentiation processes [46].

Despite these exciting discoveries, there is much that still requires elucidation, such as how biomechanical para-

meters are involved in tumour resistance to therapy. An intriguing possibility would be to take the described co-culture models and add experimentally the relevant mechanobiological elements. With respect to biomaterials and cancer it must be emphasized that patients requiring biomaterial applications are often older and thus have a decreased regenerative capacity. Moreover, they are also generally multimorbid, that is, there are other significant diseases from which they suffer, this generally meaning that they are under multiple medication. How age, other disease states, post radio- and chemotherapy and other medication affect the tissue reactions to biomaterials for cancer is a field which is currently extremely poorly, if at all, understood.

In conclusion, especially with respect to mechanobiology and tumour cell behaviour there is still an ocean of the unknown which requires to be chartered by the scientific and medical communities. It is abundantly evident that this can only be achieved by an interdisciplinary approach. In this endeavour there can be no doubt that the physicist and the engineer have invaluable contributions to make to the long-standing cooperation between biologist and clinician.

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