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ON THE FOUNDATIONS OF CANCER MODELLING: SELECTED TOPICS, SPECULATIONS, AND PERSPECTIVES

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This paper presents a critical review of selected topics related to the modelling of cancer onset, evolution and growth, with the aim of illustrating, to a wide applied mathematical readership, some of the novel mathematical problems in the field. This review attempts to capture, from the appropriate literature, the main issues involved in the modelling of phenomena related to cancer dynamics at all scales which characterise this highly complex system: from the molecular scale up to that of tissue. The last part of the paper discusses the challenge of developing a mathematical biological theory of tumour onset and evolution.

Keywords: Cancer modelling; multiscale modelling; complexity in biology; living systems.

1. Introduction

The scientific community is becoming increasingly aware that the great revolution of this century is going to be the mathematical formalisation of phenomena in the Life Sciences, much as the revolution of the past two centuries was the development of the above approach in the Physical Sciences.

To quote J. E. Cohen:

— *Mathematics is Biology's next microscope, only better*; *Biology is Mathematics' next Physics, only better.*

This endeavour is an enormous challenge that will require the intellectual energy of scientists working in the field of mathematics and physics collaborating closely with biologists and clinicians. This essentially means that the heuristic experimental approach, which is the traditional investigative method in the Biological Sciences, should be complemented by the mathematical modelling approach. The latter can be used as a hypothesis-testing and indeed, hypothesis-generating, tool which can help to direct experimental research, while the results of experiments help to refine the modelling. The goal of this research is that, by iterating back and forth between experiment and theory, eventually a deeper conceptual understanding is reached of how the highly nonlinear processes in biology interact. The ultimate goal in the clinical setting is to use mathematical models to help design therapeutic strategies.

The analysis of complex biological systems by a mathematical approach is motivated by top level biologists and is documented in several recent papers appearing in journals dedicated to the life sciences. Among others, Antia, Ganusov, and Ahmed[17] analyse the role of mathematical models in biology, while May[137] analyses relatively more general aspects of the use of mathematics in the biological sciences. This interesting paper looks for an equilibrium between a naive enthusiastic attitude and unreasonable scepticism. The beginning of the above cited paper captures the main conceptual ideas:

— *In the physical sciences, mathematical theory and experimental investigation have always marched together. Mathematics has been less intrusive in the life sciences, possibly because they have been until recently descriptive, lacking the invariance principles and fundamental natural constants of physics.*

Moreover, the same author also reports the sentiments of the great Charles Darwin:

I have deeply regretted that I did not proceed far enough at least to understand something of the great leading principles of mathematics; for men thus endowed seem to have an extra sense.

Key comments on interdisciplinary approaches can be found in various papers authored by scientists operating in the field of molecular and cellular biology. The paper by Hartwell *et al.*,[103] for example, deeply analyses the conceptual differences between the difficulties in dealing with inert and living matter. Living systems are characterised by specific features absent in classical mechanics: such as, for example, reproduction, competition, cell cycle, and the ability to communicate with other entities.

Also in this case, it is pertinent to quote from the above article:

Biological systems are very different from the physical or chemical systems analysed by statistical mechanics or hydrodynamics. Statistical mechanics typically deals with systems containing many copies of a few interacting components, whereas cells contain from millions to a few copies of each of thousands of different components, each with very specific interactions.

Although living systems obey the laws of physics and chemistry, the notion of function or purpose differentiates biology from other natural sciences.

Moreover:

More important, what really distinguishes biology from physics are survival and reproduction, and the concomitant notion of function.

It is worth stressing that essentially analogous concepts are proposed in the paper by Reed[168] according to the viewpoint of applied mathematicians. Once more, the author comments on the crucial difference between dealing with living matter and inert matter: essentially the lack of background models to support the derivation of mathematical equations.

Furthermore, along with environmental factors, survival or reproduction in living matter is determined by gene expression and resulting phenotypic characteristics. Genetic instability causing random gene mutations can alter phenotypic expression, which may increase viability or proliferation in certain environmental circumstances, thus causing the expansion of the mutation in the population. The expansion of a particular phenotype may alter the environment, which in turn affects evolutionary selection, and so on. Therefore, a particular challenge to mathematical modellers is to properly include this dynamic genetic and evolutionary component in any theoretical framework.

Cancer modelling has, over the years, grown immensely as one of the challenging topics involving applied mathematicians working with researchers active in the biological sciences. The motivation is not only scientific as in the industrial nations cancer has now moved from seventh to second place in the league table of fatal diseases, being surpassed only by cardiovascular diseases. Indeed, the World Health Organisation estimates that at present, cancer kills approximately six million people annually. Furthermore, as the population in western countries ages, for instance in the near future there will be more people in Europe over 60 than under 20, age-related illnesses, such as cancer, will become even more of a problem. For these reasons the fight against cancer is of major importance for public health (and also economic resources) throughout the world. Technical data and websites are reported in Sec. 1 of the paper by Roose, Chapman and Maini.[170]

The importance of examining the genetic mutations in cancer development is emphasised in Hanahan and Weinberg's landmark paper,[102] *The Hallmarks of Cancer*. In this paper, they identify six critical changes in cell physiology that characterise malignant cancer growth. These six changes — self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis, all incorporate some aspect of genetic mutation and evolutionary selection leading to malignant progression. Although preliminary work on cancer modelling has included one or more of these hallmarks, few theoretical papers have addressed the mutations and selection which lead to the outward expression of these characteristics. Indeed, it is well accepted that the onset of cancer occurs through a sequence of genetic

mutations and evolutionary selection leading to malignancy, a concept not yet well addressed through mathematical modelling.

Thusfar, mathematical models have mainly concentrated on only two of Hanahan and Weinberg's hallmarks: angiogenesis and invasion. Some models have incorporated other hallmarks (such as limitless replicative potential), without actually examining the mutations or environments leading to such phenotypes. As such, although the current models provide a solid foundation for the study of these cellular processes, given that these processes interact, the next step is to develop an integrated model which can then be used to investigate fully the effects of manipulating various components. Although a greater understanding of the biological pathways that lead to these physiological changes will help to create more realistic models, there are still many general questions that can be examined through modelling. Integrated, multiscale models that address each of the hallmarks of cancer, and provide mechanistic explanations for observed behaviour, could potentially provide breakthroughs in understanding cancer and improving treatment.

Modelling and simulation of tumour growth is certainly one of the challenging frontiers of applied mathematics which could have a great impact both on the quality of life and the development of the mathematical sciences. It is true that mathematics alone cannot solve the problem of cancer. However, applied mathematics may be able to provide a framework in which experimental results can be interpreted, and a quantitative analysis of external actions to control neoplastic growth can be developed. Specifically, models and simulations can reduce the amount of experimentation necessary for drug and therapy development. Moreover, the mathematical theory developed might not only provide a detailed description of the spatiotemporal evolution of the system, but may also help us understand and manipulate aspects of the process that are difficult to access experimentally.

Research perspectives in this specific field are analysed by Gatenby and Maini,[93] who suggest the development of a research line which may be called *mathematical oncology*. Indeed, applied mathematicians are becoming more and more involved in the above challenging research field. This activity is documented in a large number of mathematical research and review papers and in the collection of surveys in books[1,167,25] or special issues of journals.[30–32]

It is interesting to observe that the aforementioned cited paper[93] suggests the development of a new game theory as a fundamental paradigm to deal with the above topic. Several scientists propose this type of reasoning on the modelling of evolutionary games, as documented in the papers by Nowak and Sigmund[149] and Gatenby *et al.*[96] This topic will be more fully analysed in Sec. 5 as it may act as a fundamental paradigm towards the development of a bio-mathematical theory of cancer, that is the very final aim of the interaction between the biological and mathematical sciences in the research field under consideration.

This paper presents a review and critical analysis of some selected issues related to the mathematical approach to the modelling of phenomena in cancer, to offer to applied mathematicians suitable research perspectives with particular focus on the

following key issues:

- Multiscale aspects of the biological phenomena and of the related mathematical approaches.
- Strategies to select the correct mathematical framework to deal with modelling at each scale, being aware that the existing mathematical approaches have not yet reached the identification of a uniquely defined approach.
- Development of methods to link models at the scale selected with those at the corresponding lower and higher scales. In the case of this paper, models at the cellular scale are supplemented by models at the molecular (genetic) scale and generate models at the higher, tissue level, scale.
- Looking for paradigms for the development of a mathematical biological theory related to the complex system we are dealing with. In other words, it is worth understanding how far scientists are from the development of a mathematical theory analogous to physical mathematical theories developed in the past century.

It is worth stressing that we do not aim to cover the whole variety of issues and the vast literature in the field, but simply to capture, out of the essential bibliography on the above selected topics, the main issues related to the modelling of cancerous phenomena with special focus on multiscale aspects. Therefore, while we do not claim to carry out a complete review, we hope to cover sufficient material to motivate more applied mathematicians to develop a research activity in the field which, as we will point out, also includes highly challenging analytic problems.

We have attempted to describe problems (rather than write equations) to identify the mathematical structure of the various classes of models. The reader interested in details is guided to the specific literature by citations attempting to cover the various issues dealt with in the paper. Moreover, we do not claim completeness of bibliographical citations which have been limited to 200 titles. It is a selection out of a large and rapidly growing bibliography in the field.

After the above preliminaries, the contents of this paper, which is organised into six sections, are as follows:

— Section 2 deals with a phenomenological description of the aspects of the biology which are the subject of the various mathematical modelling approaches reviewed in the sections which follow. The contents focus on two main issues: the observation and representation scales and the implications for the mathematical approaches due to the fact that we are dealing with a living system. As we shall see, both aspects play a crucial role in the selection of the mathematical structures to be used for modelling.

— Section 3 deals with modelling at the microscopic scale, describing the evolution of cells organised into several cell populations each characterised by different biological functions and activities. Specifically, the analysis refers to two classes of models: models where all cells are equivalent, with internal structure which

may evolve in time; and models in which characteristics of the cells are allowed to vary from cell to cell and are modified by cellular interactions and signalling.

— Section 4 deals with modelling at the macroscopic scale when tumour cells are organised into solid tissues with growing and invasive competence. The appropriate modelling approach, using continuum or individual based models, depends on the system of interest. Continuum models use systems of partial differential equations corresponding to different cell populations or chemical substances. Different approaches, which are generated by different modes of closing conservation equations by phenomenological models describing the material behaviour of the system, are detailed. A fundamental problem of continuum modelling is that it tracks the average behaviour of cells, while the driving macroscopic or malignant behaviour may originate away from the average behaviour. In this case, the utilisation of discrete, cell-based models can be more appropriate. This section also reports on the link between the microscopic and macroscopic scales, through the derivation of macroscopic equations from the underlying microscopic description developed at the cellular scale.

— Section 5 reports the existing literature on the passage from the molecular to the cellular scale, i.e. from genotypic to phenotypic distributions. Then, starting from this overview, some perspective ideas are proposed in view of the last part of the paper to develop a mathematical theory for multicellular systems which include genetic mutations, onset of neoplastic cells, and growth, if not suppressed by the immune system or specific therapeutical actions.

— Section 6 presents an overview of complexity analysis related to modelling the overall system and provides a review of some recent approaches to multiscale modelling. This is a challenging and still open mathematical problem.

— Section 7 proposes a critical analysis on the background, however difficult, objective of developing a proper mathematical theory of biological systems: not simply models, but a self-consistent mathematical description, where the parameters of the models are derived from appropriate experimental data.

This paper aims to provide not only a review of the existing literature, but also a critical analysis addressed to indicate, and hopefully to provide direction on, new theoretical research perspectives. We are convinced that the complexity of the system we are dealing with requires the invention of new mathematical methods, or at least new ideas, to place into a mathematical context the above-mentioned complexity. Indeed, the above topics generate interesting and very challenging mathematical problems. Although their analysis may not, in some cases, have an immediate impact on biology, mathematicians are however attracted by them.

This is an additional aspect of the interplay between mathematics and biology: in this case biologists may be disappointed by the attraction of mathematicians towards challenging mathematical problems, even when the relevance to applications is not evident. On the other hand, mathematicians should not be blamed: at least in some cases, this analysis leads to results which are useful for various different

fields of the applied sciences and hopefully also to the progress of the mathematical sciences. In this respect, mathematicians are no different from the biologists who become engrossed in technical experimental detail and lose sight of the overall goal. Indeed, one may argue that it is essential for researchers in each discipline to do exactly that, while it is the responsibility (and the art) of interdisciplinary research to see how different techniques from different disciplines may be used to answer the overarching scientific questions.

2. Multiscale Aspects of Cancer Modelling

The complex biological system dealt with in this paper needs, as already mentioned, a multiscale mathematical approach. However, scaling[190] is not the only problem as modelling also needs a deep understanding of the different functions expressed at the different scales: from genes to biological tissues. This section is devoted to a brief preliminary analysis of some biological phenomena related to the aforementioned scales.

While the description we provide may not be satisfactory from the viewpoint of biology, it attempts to extract some interpretations of reality to set the essential background for the mathematical approach reviewed in what follows. The reader, interested in a deeper understanding of the biology of cancer, will find the book by Weinberg[197] a highly valuable reference.

The first event to be considered is the generation of a neoplastic cell through phenotypic alterations resulting from genetic mutations occurring through genetic instability and environmental interactions. After the onset of neoplasia, various complex phenomena occur which are related to different scales. The characterisation of the system suggests the identification of three *natural scales* which are also connected to different stages of the disease: processes on the *cellular scale* are triggered by signals stemming from the *sub-cellular level* and have an impact on the *macroscopic scale*, i.e. on the organism, when tumours grow and spread. In detail:

The sub-cellular scale: The evolution of a cell is regulated by the genes contained in its nucleus. Receptors on the cell surface can receive signals which are then transmitted to the cell nucleus, where the aforementioned genes can be activated or suppressed. In extreme situations, particular signals can induce uncontrolled cell proliferation, or cell death- so-called apoptosis or programmed cell death. Unregulated proliferation may activate interactions between tumour cells and host cells, which occur at the cellular level but are mediated by subcellular processes, such as through signal cascades and receptor expression. These interactions can result in temporary, or even permanent, alterations in gene expression, which in turn can affect a cell's state, such as activation or inactivation of immune cells.

The cellular scale: On the microscopic level, models are proposed to simulate the effects of cell–cell interactions. These interactions are key elements at all stages of tumour formation, whether they are among tumour cells and host cells, or among

tumour cells themselves. For example, early in tumour development, if the immune system is active and able to recognise tumour cells, it may be able to develop a destruction mechanism and induce cancer cell death; otherwise, the tumour may evade apoptosis or co-opt the host cells, allowing progressive growth. During invasion and metastasis, alterations in cell–cell adhesion between individual tumour cells are key to driving the process. These and other cellular interactions are regulated by signals emitted and received by cells through complex transduction processes. Therefore, the connection to the aforementioned sub-cellular scale is evident. On the other hand, the growth of tumour cells, if not cleared by the immune system, will form a mass so that macroscopic features become important. However, even after the formation of a tumour structure, interactions between individual cells (signalling, migration or proliferation) are crucial to driving macroscopic processes (such as blood vessel formation or invasion), underscoring the need to link these multiple scales.

The macroscopic scale: The initial developing tumour can be characterised by *three zones*: an external proliferating layer, an intermediate layer in which there are clusters of quiescent tumour cells, and an inner zone with necrotic cells. Prior to vascularisation, these avascular tumours reach an equilibrium size of about 2 mm in diameter, where their growth is limited by diffusion of nutrients until the onset of angiogenesis (the process of formation of new blood vessels, induced by factors secreted by the tumour, and vital for continued tumour growth). Although the angiogenic process is often described macroscopically, it occurs through processes at the cellular scale, such as migration, proliferation, and cell–cell signalling. These events are generated at the gene level. The above description of tumour evolution can also occur with different geometrical characteristics, for instance around cords or surfaces bounding nutrient sources. In these cases, the proliferative zone will be (internal) next to the nutrient supply and the necrotic zone will be the outermost layer.

After the initiation of the "angiogenic switch", the tumour expands and becomes a heterogenous tissue, where some of the previously mentioned "layers" may actually occur as "islands", leading to a tumour comprised of multiple regions of necrosis, engulfed by tumour cells in a quiescent or proliferative state. Furthermore, these zones vary through the course of tumour development, as nutrient delivery alters via blood vessel collapse or formation, changing proliferating zones to necrotic and vice versa. In each of these layers, interactions between tumour and normal cells (such as immune cells and blood vessels) are crucial in determining growth and malignant progression. Here, one has the overlap of phenomena at the cellular level with typical macroscopic behaviour such as diffusion or, more generally, phenomena that can be related to the mass balance or evolution of macroscopic variables such as tumour size. Moreover, most transitions from one cellular state to another are induced at the genetic level.

Different mathematical methods and structures correspond to the different scales described above. For instance, models at the cellular scale are generally developed in terms of ordinary differential equations or Boolean networks,

while multicellular systems are modelled by nonlinear integro-differential equations similar to those of nonlinear kinetic theory (the Boltzmann equation), by individual-based models which give rise to a large set of discrete equations, or by partial differential equations for systems with internal structures. Macroscopic models lead to systems of nonlinear partial differential equations or discrete modelling approaches. Nonlinearity is an intrinsic feature of all models. However, the above introduction to mathematical structures needs to be put into a detailed mathematical framework, and this is the main focus of the sections which follow.

3. Modelling at the Cellular Scale

Section 2 illustrates that different scales are needed to represent crucial phenomena occurring during tumour growth. Moreover, it may be that all scales are needed to understand various biological phenomena. Hence, multiscale methods should be developed and the following strategy is one possibility: identify the mathematical structures needed to describe biological phenomena at each scale, then connect the various structures to model the overall system, viewing it as a network of several interconnected subsystems or modules with feed-back down and feed-forward up scales.

This section is devoted to modelling at the cellular scale as the intermediate between the molecular and the macroscopic scales. The passage from the lower to the higher scale will be treated in Secs. 4 and 5.

Multicellular systems have been modelled by different approaches corresponding to different levels of approximation of biological reality. Each approach needs different mathematical structures as documented in the three subsections which follow corresponding, respectively, to population dynamics, populations of cells with internal structure, and kinetic theory for active particles. Then, in Sec. 4, a short discussion and critique is given.

Concerning the aforementioned different modelling approaches, it is important to comment on their ability to model heterogeneous phenomena, i.e. the non-uniform distribution of the microscopic states (biological functions) of cells. A schematic of heterogeneity is given in Fig. 1, representing different stages and distributions of mutated cells which have lost their differentiation state and are progressing towards metastatic competence. In this case the biological function expressed by cells is called *progression.*[100,150]

The following review is limited to the indication of mathematical structures which may act as general paradigms for the derivation of specific models to be cast into these frameworks. Details on specific models can be obtained by reference to the bibliography.

3.1. *Population dynamics*

Coupled ordinary differential equations can be used to model large systems of cell populations, where each variable corresponds to a well-defined biological property

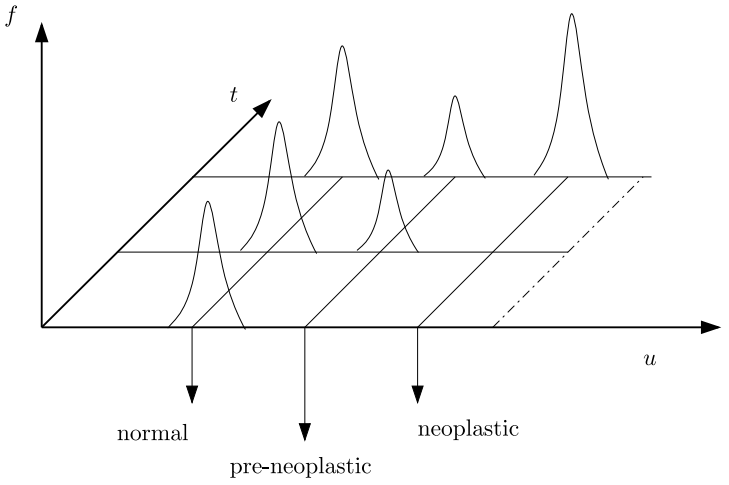

Fig. 1. Heterogeneity and progression of tumour cells: Time evolution of the probability distribution (f) over the variable (u) expressing the differentiation state of cells.

characteristic of all cells of the same population. These models are formulated by averaging over the space variable and over the biological function expressed by each population so that the state of the system is simply described by the number density of cells within each population, while their structure is a technical development of Lokta–Volterra type equations:

$$\frac{dn_i}{dt} = \varphi_i(t, \mathbf{n}), \tag{3.1}$$

where $\mathbf{n}$ denotes the set $\{n_1, \ldots, n_n\}$ of the numeric densities of cells, for $i = 1, \ldots, n$, for a system of n interacting populations.

The structure of the terms φ_i depends on the modelling approach related to the specific system under consideration. The presence of time in the argument may appear if the system is open to external time-dependent actions, e.g. chemotherapy.

The literature in the field is vast, developed from pioneering papers modelling the onset of cancer and the temporal evolution of cell density supported by the presence of nutrients, but limited by competition with the immune system. The paper by Gyllenberg and Webb,[101] to our knowledge, initiated a systematic development of population dynamics models focused on cancer. This approach has been developed by various authors as documented in the collection of surveys by Adam and Bellomo[1] and in the bibliography of the various papers published in special issues of journals.[109,117] The book by Perthame[158] provides, in Chap. 1, a survey of population models in biology, related to a vast bibliography documented in several books, e.g. Edelstein-Keshet[77] and Thieme.[185] The review is completed by an introduction to classical mathematical problems: stability analysis, bifurcation and asymptotic behaviour.

Various authors have used the above approach to model open systems subject to different therapies, e.g. Kirschner and Panetta,[115] Nani and Freedman,[148] d'Onofrio,[71] De Pillis *et al.*,[65,66,68] Arciero *et al.*,[18] Moore and Li,[147] Byrne *et al.*[47]

Tomlinson and Bodmer,[187] d'Onofrio and Tomlinson,[72] Johnston *et al.*[112,113] deal with a deeper additional insight into cell dynamics modelling failure of programmed cell death and differentiation as causes of tumour growth. Smieja and Swierniak[179] develop models of chemotherapy based on gene amplification analysis.

A detailed report on the application of population dynamics models in immunology is given in the review paper by Perelson and Weisbuch[157] related to biological theory in the field.[39,75,87,156]

Time delay is introduced by various authors, e.g. Foris and Bodmer,[82,83] to model how the delayed response of the immune system in identifying the presence of tumour cells acts as a bifurcation parameter to separate two different outputs of competition: blow-up of tumour cell density or its suppression due to the action of the immune system.

The advantage of the above approach is that models are easily tractable, allowing a relatively rapid identification of the parameters characterising the model by suitable comparisons with experimental data. On the other hand, these simplifications omit potentially important phenomena, such as spatial aspects, and heterogeneity[100] among cells of the same population. As we shall see, the latter plays a potentially important role in the evolution of the system and the various interactions and competitions between cells of different populations. Moreover, the identification of parameters is definitely useful for application, but it is at the macroscopic scale so that it cannot be easily related to specific biological functions generally developed at the molecular and cellular scale.

Finally, it is worth discussing the concept of *populations* with reference to the idea of *functional modules* proposed by Hartwell *et al.*[103] After having observed that biological systems may be characterised by an enormous number of copies, while only a few copies generally characterise living systems, it is proposed[103] to reduce complexity by grouping different entities through looking at their collective expression of biological functions related to the specific biological events under observation. This suggestion is developed[29] in a way that each population can be regarded as a module.

3.2. *Population dynamics with internal structure*

Systems of partial differential equations can be used to model large systems of interacting cells whose microscopic state includes internal variables related to biological functions. This internal structure, generally a scalar variable, characterises specific functions of the cell and can greatly influence the biological events under consideration. For instance, the internal variable can be the age of the cell as determined by the cell cycle, which has crucial influence on various biological phenomena such as apoptosis, cell division, mutation, etc.

A systematic introduction to cell population dynamics with internal structure has been given by Webb,[196] followed by several interesting books, e.g. Iannelli,[110] Cushing,[60] Dieckmann and Heesterbeck,[69] Thieme,[185] Perthame,[158–160] Perthame and Zubelli,[162] Dyson *et al.*[76] The bibliographies cited in these monographs cover the existing literature in the field.

Age-structured models describe the evolution of cellular systems for times of the same order of the cell cycle. Their structure is as follows:

$$\partial_t N_i(t,a) + \partial_a N_i(t,a) = Q_i(\mathbf{N})(t,a), \tag{3.2}$$

where $\mathbf{N}$ denotes the set $\{N_1, \ldots, N_n\}$ of the cell densities for $i = 1, \ldots, n$, of a system of n interacting populations of cells. The densities N_i depend on time and on the age a. The terms Q_i refer to interactions between populations. Note that a further complication in these models is that if, for example, a is "age" taken as progression through the cell cycle, then we also need to know how this evolves in time, as this will alter the form of the above equation.

3.3. *On the kinetic theory for active particles*

Biological function is statistically distributed across cells. This biological phenomenon generates the so-called *heterogeneity* related to *progression* and to *immune activation* as documented, for example, in the papers by Greller, Tobin and Poste[100] and Nowell.[150] Models should have the ability to describe progression of cancer cells and their competition with immune cells which express their antagonistic ability, unless inhibited, to limit cancer cell density growth.

Particular pathologies may be thought of as an emergent property of the output of various genetic mutations which generate new cells with increasing degree of malignancy. After various genetic mutations, cells may acquire the ability to succeed in escaping from the immune system despite the sentinel guards which, should, in principle, limit cell growth. (See, for example, Lollini, Motta, Pappalardo and Castiglione,[130,124] Vogelstein and Kinzler,[192] Blankenstein[39]).

Application of mathematical kinetic theory to model immune competition with specific focus on cancer was initiated by Bellomo and Forni[27] and subsequently developed by various authors. Recent contributions are due to, among others, Derbel,[64] De Angelis and Jabin,[61] Kolev,[118] Kolev, Kozlowska and Lachowicz,[119] Bellouquid and Delitala,[33,34] Brazzoli and Chauviere.[43] Several interesting results are reported in the already cited book by Bellouquid and Delitala[35] which, in particular, address a number of aspects of the above-mentioned competition.

Types of biological function differ from population to population, while the overall representation of the multicellular (multi-population) system is statistically described by the distribution functions:

$$f_i = f_i(t,u) : \quad [0,T] \times D_u \to \mathbb{R}_+, \quad i = 1, \ldots, n, \tag{3.3}$$

over the microscopic state $u \in D_u$ of cells of each population labelled by the subscript i.

By definition, $dn_i = f_i(t,u)\,du$ denotes the number density of cells, regarded as active particles, which, at time t, are in the element $[u, u+du]$ of the space of the microscopic state. Mathematical models should then describe the evolution in time of the distribution functions f_i. When these functions are obtained by solution of the resultant equations gross averaged quantities can be computed. For instance, the local *number density* of cells is calculated, under suitable integrability assumptions on f_i, as follows:

$$n_i(t) = \int_{D_u} f_i(t,u)\,du, \tag{3.4}$$

while the following quantities:

$$a_i = a[f_i](t) = \int_{D_u} u f_i(t,u)\,du \tag{3.5}$$

and

$$A_i = A[f_i](t) = \frac{a[f_i](t)}{n_i(t)} \tag{3.6}$$

have been called,[29] respectively, *activation* and the *activation density*. These quantities represent, respectively, the overall activity of the cells per unit volume and their mean activity. Analogous calculations can be developed for higher order moments.

The formal structure, which describes the evolution of f_i, is obtained by the balance of particles in the elementary volume of the microscopic state. Only the case of spatial homogeneity is reported in what follows. Indications will then be given as to how to deal with models with spatial structure. The following interactions are considered:

- External actions, either therapeutical actions or other external agents, which modify the distribution function;
- Stochastic modification of the microscopic state of cells due to binary interactions with other cells of the same or of different populations. These interactions are called *conservative* as they do not modify the number density of the various populations;
- Genetic alteration of cells which may either increase the progression of tumour cells or even generate, by clonal selection, new cells in a new population of cancer cells with higher level of malignancy;
- Proliferation or destruction of cells due to binary interactions with other cells of the same or of different populations.

Consequently one has:

$$\partial_t f_i(t,u) + \mathcal{F}_i(t)\,\partial_u f_i(t,u) = J_i[f](t,u) = C_i[f](t,u) + P_i[f](t,u) + D_i[f](t,u), \tag{3.7}$$

where the right-hand side models the flow, at time t, into the elementary volume $[u, u+du]$ of the state space of the ith population due to transport and interactions.

In detail:

- $\mathcal{F}_i(t)$ models the external action over the ith population.
- $C_i[f](t,u)$ models the flow, at time t, into the elementary volume of the state space of the ith population due to conservative interactions:

$$C_i[f](t,u) = \sum_{j=1}^{n} \eta_{ij} \int_{D_u} \int_{D_u} \mathcal{B}_{ij}(u_*, u^*; u) f_i(t,u_*) f_j(t,u^*)\, du_*\, du^* - f_i(t,u) \sum_{j=1}^{n} \eta_{ij} \int_{D_u} f_j(t,u^*)\, du^*, \tag{3.8}$$

  where η_{ij} is the encounter rate, namely, the encounters of a *candidate particle*, with state u_* in the ith population, with a *field particle*, with state u^* in the jth population. The probability that, as a result of this interaction, the particular acquires the state u is given by the probability density function $\mathcal{B}_{ij}(u_*, u^*; u)$.
- $P_i[f](t,u)$ models the flow, at time t, into the elementary volume of the state space of the ith population due to proliferation:

$$P_i[f](t,u) = \sum_{h=1}^{n} \sum_{k=1}^{n} \eta_{hk} \int_{D_u} \int_{D_u} \mu_{hk}^{i}(u_*, u^*; u) f_h(t,u_*) f_k(t,u^*)\, du_*\, du^*, \tag{3.9}$$

  where $\mu_{hk}^{i}(u_*, u^*; u)$ models the net proliferation into the ith population, due to interactions, which occur with rate η_{hk}, of the *candidate particle*, with state u_*, of the hth population and the *field particle*, with state u^*, of the kth population.
- $D_i[f](t,u)$ models the net flow, at time t, into the elementary volume of the state space of the ith population due to proliferative and destructive interactions without transition of population:

$$D_i[f](t,u) = \sum_{j=1}^{n} \eta_{ij} \int_{D_u} \mu_{ij}(u_*, u^*, u) f_i(t,u_*) f_j(t,u^*)\, du_*\, du^*, \tag{3.10}$$

  where $\mu_{ij}(u_*, u^*, u)$ models net flux within the same population due to interactions, which occur with rate η_{ij}, of the *test particle*, with state u, of the ith population and the *field particle*, with state u^*, of the jth population.

Substituting the above expression into (3.7), in the absence of external action, yields:

$$\frac{\partial}{\partial t} f_i(t,u) = \sum_{j=1}^{n} \eta_{ij} \int_{D_u} \int_{D_u} \mathcal{B}_{ij}(u_*, u^*; u) f_i(t,u_*) f_j(t,u^*)\, du_*\, du^* - f_i(t,u) \sum_{j=1}^{n} \eta_{ij} \int_{D_u} f_j(t,u^*)\, du^*$$

$$+\sum_{h=1}^{n}\sum_{k=1}^{n}\eta_{hk}\int_{D_u}\int_{D_u}\mu_{hk}^{i}(u_*,u^*;u)f_h(t,u_*)f_k(t,u^*)\,du_*\,du^*$$
$$+\sum_{j=1}^{n}\eta_{ij}\int_{D_u}\mu_{ij}(u_*,u^*,u)f_i(t,u_*)f_j(t,u^*)\,du_*\,du^*. \tag{3.11}$$

The above structure acts as a framework for the derivation of specific models, obtained by a detailed consideration of microscopic interactions. The modelling needs to be completed to generate well-defined expressions for the terms η, $\mathcal{B}$ and μ. However, following the principle that we must try, where possible, to reduce complexity, each population is identified only by its ability to perform one biological function (activity).

Models of spatial dynamics can be developed by different approaches. For instance Bellouquid and Delitala[35] model cell–cell interactions by assuming they depend on the distance between and microscopic states of the interacting pairs. A relatively simpler approach has been proposed by Othmer and Hillen[152] to derive equations at tissue level from the underlying microscopic description. Models with spatial structure are obtained by a random walk perturbation of spatially homogeneous models.

3.4. *Summary*

Various mathematical approaches to modelling multicellular systems at different representation scales have been reviewed in this section. The advantages of each approach with respect to the others have already been discussed. However, if we wish to model at a certain scale, *a priori*, we have no indication of which other scales we must take into account. In most cases, one probably has to consider many scales. In reality, the choice will be determined by the question we are trying to answer and the availability of data and biological insight.

Moreover, the above review does not cover the whole variety of methods. Among others, one may consider individual-based models (Othmer, Dumbar and Alt,[151] Stevens[181]) which deal with the dynamics of individual cells (see also Drasdo *et al.*[73] and Drasdo and Home[74]). Hybrid models can be developed by using cellular automata or agent-based approaches,[67] where the macroscopic flow is linked to the microscopic biological state of cells. This type of framework appears more natural for the inclusion of individual cell properties and therefore is conceptually more straightforward. However, as the number of cells increases to biologically realistic levels, it can become computationally intractable.

Hybrid approaches have been proposed[53] based on population dynamics models with stochastic coefficients arising from modelling kinetic type interactions at the molecular level. These approaches, which are still in the early stages of investigation, have the potential to reduce significantly the complexity of models of the kinetic theory for active particles.

The above reasoning shows that when a high level of accuracy of description is reached, not only is computational complexity involved, but there is also the implicit difficulty of determining the parameters of the model. Therefore, the problem of selecting the most appropriate modelling approach is still open.

4. From Cellular to Macroscopic Tissue Models

A large body of literature has been devoted to models which link the cellular scale to the macroscopic tissue scale. In this way, models can address how changes in cell–cell interactions affect the macroscopic properties of the tumour. Once cells have formed a tumour mass, features on the macroscopic level of the tumour environment must be considered. For example, the availability of nutrients and transport of cytokine signals has a profound impact on tumour progression. Conversely, the tumour cells themselves can create areas of acidosis which in turn affect the properties of the cellular and acellular components of the surrounding environment.

Models which address how cellular changes affect macroscopic distributions are especially important when examining Hanahan and Weinberg's last two hallmarks of cancer: sustained angiogenesis, and tissue invasion and metastasis.[102] Also, it is important to note that although these macroscopic properties can be sufficiently modelled at a single scale, they occur through genetic mutations and evolutionary selection. This link has not yet been fully modelled, and will be discussed in Sec. 5.

Nevertheless, there has been much success in using macroscopic models to examine tumour malignancy. The two main types of models used are continuum, which examine average behaviour of the densities of populations or components, and discrete, which can track the behaviour of individual cells. These types of models employ a wide variety of mathematical methods, as they can describe phenomenological interactions between cells or mechanical interactions based on measuring stresses and strains of the system. All of these methods make some *a priori* assumptions about cell behaviour: for example, they either assume a cell moves through a process like diffusion, or the cellular components act like an elastic fluid. There have been some attempts at validating these assumptions mathematically, by building cellular models and extrapolating movement behaviour at the macroscopic scale, which we will also discuss in Sec. 4.3.

Figures 2 and 3 illustrate schematically some important points that must be incorporated in a continuum description. Figure 2 illustrates tumour-generated substances (angiogenic factors) which attract endothelial cells towards the tumour. These cells form blood vessels so at the macroscopic level one sees capillary sprouts moving towards the tumour to provide nutrient and allow further growth.

Figure 3 shows in more detail the structure of a tumour representing the inner zone (dark) of necrotic cells, the intermediate one (light) of quiescent cells, and the proliferative outer zone (grey). Chemoattractants are generated by the hypoxic zone near the necrotic zone.

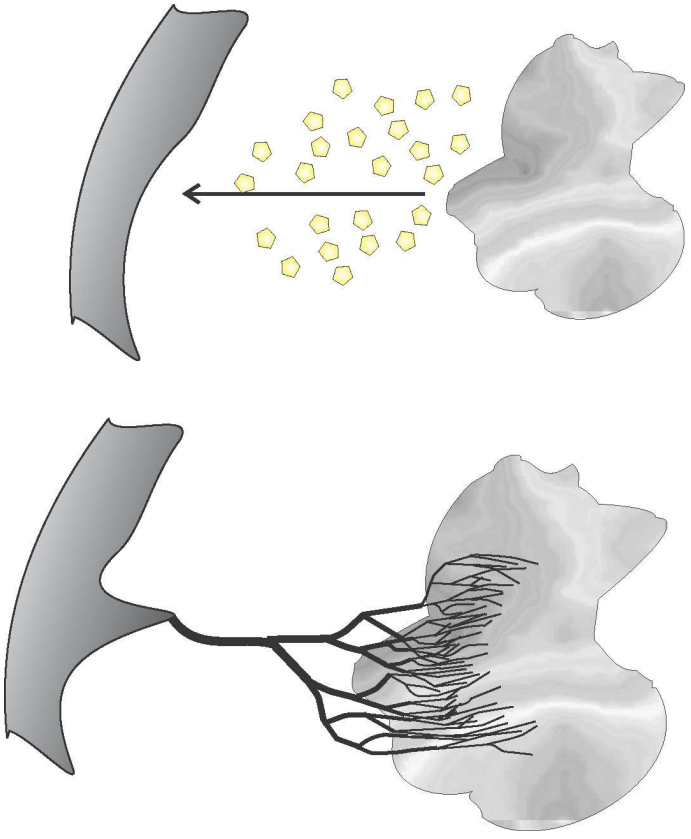

Fig. 2. Schematic of tumour-induced angiogenesis. Upper panel: generation of chemical substances, (pentagons) by the tumour mass (on the right) attract blood vessels (lower panel), with genesis of capillary sprouts from existing vasculature to feed the tumour and allow further growth.

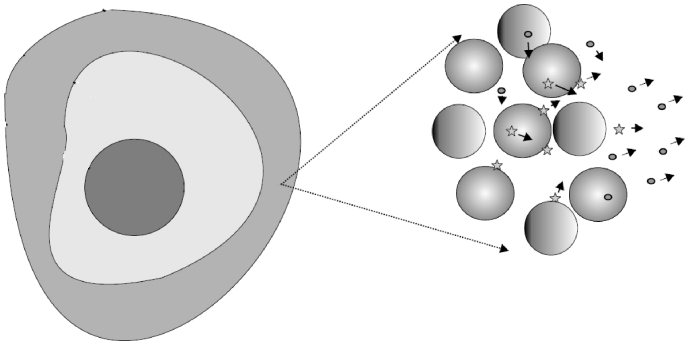

Fig. 3. Schematic of growing tumour illustrating the different biological phases: necrotic, quiescent, and proliferative cells with generation of chemoattractants. Cells communicate among themselves, and with the outer environment, by signalling and chemical factors (stars and circles) that move (as shown by arrows) in the environment.

4.1. *Mathematical framework for continuum models*

Continuum models at the macroscopic scale are generally stated in terms of partial differential equations which describe the evolution in time and space of locally averaged quantities related to the behaviour of cell populations. A large variety of continuum models are derived using mass balance equations for the cellular components and reaction–diffusion equations for the chemicals or nutrients. However, different choices of the mathematical form of the cellular movement lead to very different models, which we will explore presently.

The initial system is composed of mass balance equations for the cellular components, extracellular matrix (ECM), and the extracellular fluid (ECF), Eq. $(4.1)_1$, coupled to a system of reaction-diffusion equations for the concentration of extracellular chemicals, Eq. $(4.1)_2$.

$$\begin{cases} \rho_j \left[\dfrac{\partial \phi_j}{\partial t} + \nabla_{\mathbf{x}} \cdot (\phi_j \mathbf{v}_j) \right] = \Gamma_j(\rho, \phi, c), \quad j = 1, \ldots, L, \\ \dfrac{\partial c_i}{\partial t} + \nabla_{\mathbf{x}} \cdot (c_i \, \mathbf{v}_\ell) = \nabla_{\mathbf{x}} \cdot (Q_i(\rho, \phi, c) \nabla c_i) + \Lambda_i(\rho, \phi, c), \quad i = 1, \ldots, M, \end{cases} \tag{4.1}$$

where $\phi_j = \phi_j(t, \mathbf{x})$ denotes the concentration of each component, e.g. cells, matrix, or fluid, and $c_i = c_i(t, \mathbf{x})$ denotes the concentrations of the chemicals and nutrients. In Eq. $(4.1)_1$, ρ_j are the mass densities of cellular components and $\mathbf{v}_j$ is the mass velocity of the jth population while $\mathbf{v}_\ell$ is the velocity of the liquid. Moreover, $\Gamma_j(\rho, \phi, c)$ is a source term for the particular component which might include, for example, production and death terms. In Eq. $(4.1)_2$, $Q_i(\rho, \phi, c)$ is the diffusion coefficient of the ith chemical factor, and $\mathbf{v}_i$ is the velocity of the chemical component. $\Lambda_i(\rho, \phi, c)$ is the source term for the particular nutrient or chemical, which might similarly include, for example, production and uptake by the cells. Therefore, the component equations are coupled to the chemical equations via the source terms, $\Lambda_i(\rho, \phi, c)$ and $\Gamma_j(\rho, \phi, c)$. For example, blood vessels might be the source of oxygen, which is consumed by the tumour cells and in turn alters the proliferation or death rate of the tumour cells.

In some cases, the theory of mixtures is used to describe the tumour tissue.[13,50,48] The concept behind mixture theory is that at every point there is a fraction of each constituent type, unlike a system where at each spatial point there can only be one type of constituent at a time. The model then treats the tumour as a multiphase material of cells, ECM and ECF. Hence, the components are represented as volume ratios, where ϕ_j represents the volume fraction of a particular component. Sometimes the system is assumed to obey the no-voids condition, i.e. the sum of the constituents equals 1.

It is crucial to note that the system (4.1) is not a closed or self-consistent system, and therefore by itself it is not a sufficient model. Importantly, one needs to determine an equation for the velocity $\mathbf{v}_j$ in order to close the system. This leads to two main classes of macroscopic models, each defined by the choice of

movement term. These classes can be broadly defined as phenomenological models or mechanical models. Phenomenological models make an assumption about movement ignoring mechanical effects, such that cells (or matrix components) do not move, or that they move through any combination of diffusion, chemotaxis, haptotaxis, etc. In contrast, mechanical models use force-balance or momentum-balance interactions to determine how the cell, matrix and fluid components move in response to the physical forces involved. These mechanistic models take into account stresses and strains to track cellular and tissue deformation. However, these models also make assumptions about cell movement through the choice of how to model the tumour tissue, matrix and interstitial fluid (for example, treating the tumour as an elastic fluid or porous medium). In reality, it is likely that a combination of these models of motion is required, as the tumour cells probably use active motility, such as chemotaxis,[199] while at the same time responding to physical forces.

4.1.1. *Closure by phenomenological models*

In general, most of the tumour growth models are closed by phenomenological assumptions, and an explicit equation for the cellular velocity is written. These models explicitly specify the nature of the cellular movement, either unbiased or biased. Mathematical models using unbiased models often assume that cells move in response to population density. For example, early work by Ward and King,[194,195] and Bertuzzi *et al.*[37] assumed cell populations moved as a result of a single convective velocity field created through cell proliferation and death driving local volume changes.

One common example of a phenomenological closure is to assume cells move down a gradient in cell density, which leads to some type of diffusion equation for cell movement. Hence, $\mathbf{v}_j = -D_j \nabla_{\mathbf{x}} \phi_j$ where in most cases D_j is a positive constant, hence the movement is simply linear diffusion. This type of approach has proven useful in modelling the spatial spread of many types of populations. However, its applicability for interacting constituents is not as clear. Therefore, recent work has investigated the more general case of $D_j = D_j(\phi, c)$ leading to a nonlinear diffusion term.[176,186] In a paper by Sherratt,[176] a nonlinear diffusion model is used for the interactions between tumour cells and ECM. The model was able to suggest a mechanism by which some types of tumours become "encapsulated" by a highly dense ECM.

An interesting extension of this framework is used in Sherratt and Chaplain[173] where they model movement by contact inhibition in avascular tumour growth. Importantly, this model also exhibited the traditional avascular tumour structure of a proliferating rim, quiescent band, and necrotic core in terms of continuous cell densities instead of discrete bands of cell types. Previous models by Ward and King,[195] and Greenspan[99] have imposed these layers *a priori* as bands of cell types separated by moving boundaries.

Alternatively, phenomenological models can specify biased movement such as chemotaxis[54] or haptotaxis.[15,14] Future models will have the challenge of rigorously proving whether, and in which situations, tumour cells move in a haptotactic or alternate manner.

Using the above framework, numerous papers have been written to study the cellular processes driving the macroscopic properties of angiogenesis and invasion. For invasion models, travelling wave analysis is used to examine how the tumour invades the surrounding normal tissue or ECM. Gatenby and Gawlinsky[90] utilised a spatially one-dimensional continuum reaction-diffusion population competition model based on the observation that nearly all invasive tumours exhibit upregulated glycolysis (a type of anaerobic respiration which produces lactic acid) even in the presence of oxygen. They suggested that the tumour cells create an acidic environment toxic to normal tissue, and that when the normal tissue dies from the high acidity, it provides space for the tumour to proliferate and invade into the surrounding tissue. Using a set of reaction–diffusion models, Gatenby and Gawlinsky were able to predict the presence of an acellular gap in certain circumstances, which was later found experimentally.[90,92]

In contrast to the acid-invasion model, Perumpanani *et al.*[164] suggested a combination of ECM degradation by proteases and tumour cell haptotaxis as a mechanism for invasion. They performed a travelling wave analysis on a continuum 1-D ODE model of invasive cells, ECM and protease. Perumpanani and Byrne[163] extended the model to use a combination of diffusion and haptotactic movement, and found that ECM heterogeneity affects invasion.

Although these phenomenological models do an excellent job of approximating cellular motion, they fail to take into account mechanical causes of cell movement. As tumour cells proliferate, they push into the surrounding tissue, causing pressure to build. This pressure, along with other mechanical interactions, can have a very important effect on tumour growth and progression.

4.1.2. *Closure in mechanical models*

In contrast to the above modelling approaches, mechanical models close the system by specifying cell movement based on physical forces. These models aim to describe how the mechanical properties of the tumour and surrounding tissue influence tumour growth. In this framework, one would use the same mass-balance equations as in Eq. $(4.1)_1$, and continue by writing the momentum balance equations

$$\rho\phi_j\left(\frac{\partial \mathbf{v}_j}{\partial t} + \mathbf{v}_j \cdot \nabla_{\mathbf{x}}\mathbf{v}_j\right) = \mathbf{F}_j[\phi, \mathbf{v}], \quad j = 1, \ldots, L, \tag{4.2}$$

for the constituents, where $\mathbf{F}_j[\phi, \mathbf{v}]$ is a term describing the forces on the constituent j. For example, one might express $\mathbf{F}_j = \nabla_{\mathbf{x}} \cdot \mathbf{T}_j + \phi_j \mathbf{f}_j + \mathbf{m}_j$, where $\mathbf{m}_j$ is the interaction force with the other constituents, $\mathbf{T}_j$ is the stress–tensor, and $\mathbf{f}_j$ is the body force acting on the jth constituent. As before, these equations are coupled to the nutrients and chemicals in Eq. $(4.1)_2$.

The model above requires the specification of the constitutive equations relating the forces determining cell motion to the level of stress and compression. For example, as a cell undergoes mitosis and divides into two cells, the daughter cells generate a "pressure" which displaces the neighbouring cells, thus leading to an increase in tumour size. Although it is possible, as a first approximation, to describe the influence of stress on growth through continuum equations, it is clear that it is preferential to use a multiscale approach because the perception of stress exerted by a single cell and the initiation of mitosis or apoptosis occurs at a subcellular scale.

In any case, when using a mechanical continuum description, there are several different classes of mechanical models depending on whether the cells are assumed to behave like a type of fluid or solid medium. If one assumes the cells behave as a fluid, the simplest constitutive equation for the stress comes from assuming the cells act like elastic liquids

$$\mathbf{T}_j = -\Sigma_j \mathbf{I}, \tag{4.3}$$

where Σ_j is the response of the cells to compression, driving them towards regions of lower stresses. In practice, Σ_j may depend on ϕ_j.

In some special cases, the assumption of the cells moving as an elastic fluid within a rigid ECM can lead to closure by Darcy's law, where if, for example, $\mathbf{f}_j = \mathbf{0}$,

$$\mathbf{v}_j = -K\nabla_{\mathbf{x}}\Sigma_j, \tag{4.4}$$

where K is the permeability property of the matrix. This constitutive equation has two interpretations: the first is that the system acts as an over-damped force balance, the second is that the fluid-like cells flow through the rigid ECM akin to porous media flow. See Refs. 63 and 55 with reference to the closure (4.4).

Alternatively, the cell-matrix milieu can be hypothesised to be like a viscous fluid, where the stress depends on the viscosity, as in Breward, Byrne and Lewis,[44] Byrne and Preziosi,[50] and Byrne *et al.*,[48] or can be modelled as a viscoelastic fluid. Holmes and Sleeman[108] developed a mechano-chemical model of angiogenesis, modelling the ECM as a linear viscoelastic material, including chemotaxis and haptotaxis of endothelial cells. Their model was able to examine the mechanical effects of the ECM on endothelial cell migration, as well as the effects of cellular traction on the ECM deformation and resulting patterns.

Another class of models views the tumour tissue as a mixture of cells living in a porous medium made of ECM and filled with extracellular liquid, see Graziano and Preziosi.[98] Therefore, Darcy's law can be used to model both fluid flow and cell motion, considering the latter as a granular material flowing in the porous ECM scaffold. For example, one can write mass and momentum balance for the tumour cells ($j = T$) and ECF with chemicals and nutrients within it ($j = \ell$). If, for example, the mixture has no voids, so that $\phi_T + \phi_\ell = 1$, then by looking at mass and momentum balances for the whole mixture, the following conditions

must hold:

$$\Gamma_T + \Gamma_\ell = 0, \tag{4.5}$$

$$\mathbf{m}_T^\sigma + \Gamma_T \mathbf{v}_T + \mathbf{m}_\ell^\sigma + \Gamma_\ell \mathbf{v}_\ell = \mathbf{0}, \tag{4.6}$$

where $\mathbf{m}_T^\sigma$ and $\mathbf{m}_\ell^\sigma$ are the forces on the tumour and on the extracellular liquid, respectively, due to their interaction with other constituents.

The equation for composite velocity, $\boldsymbol{\xi}$, is then

$$\nabla_{\mathbf{x}} \cdot \boldsymbol{\xi} = \nabla_{\mathbf{x}} \cdot (\phi_T \mathbf{v}_T + \phi_\ell \mathbf{v}_\ell) = \mathbf{0}. \tag{4.7}$$

If the densities of the constituents are equal, then the composite velocity is equal to the mass average velocity and the momentum equation for the mixture simplifies to

$$\rho \left(\frac{\partial \boldsymbol{\xi}}{\partial t} + \boldsymbol{\xi} \cdot \nabla_{\mathbf{x}} \boldsymbol{\xi} \right) = \nabla_{\mathbf{x}} \cdot \mathbf{T}_m + \phi_T \mathbf{f}_T + \phi_\ell \mathbf{f}_\ell, \tag{4.8}$$

where $\mathbf{T}_m$ is the stress–tensor of the mixture.

In this example, as the tissue is treated as a porous medium filled with extracellular liquid, one can assume Darcy's law for the fluid flow through the tumour

$$\phi_\ell (\mathbf{v}_\ell - \mathbf{v}_T) = -K(\nabla_{\mathbf{x}} P - \mathbf{f}_\ell), \tag{4.9}$$

where K is the permeability, divided by the viscosity of the fluid, of the tissue interstitial space and P is the local interstitial fluid pressure. Neglecting inertial terms and assuming the constitutive equation

$$\mathbf{T}_m = -[P + \Sigma_T]\mathbf{I}, \tag{4.10}$$

with Σ_T positive in compression, the momentum equation for the mixture implies

$$\nabla_{\mathbf{x}} P = -\Sigma_T' \nabla_{\mathbf{x}} \phi_T + \phi_T \mathbf{f}_T + \phi_\ell \mathbf{f}_\ell, \tag{4.11}$$

where $\Sigma_T' = d\Sigma_T / d\phi_T$.

On the other hand,

$$\mathbf{v}_\ell = \mathbf{v}_T - \frac{K}{(1-\phi_T)}(\nabla_{\mathbf{x}} P - \mathbf{f}_\ell) = \mathbf{v}_T + \frac{K}{(1-\phi_T)}[\Sigma_T' \nabla_{\mathbf{x}} \phi_T + \phi_T(\mathbf{f}_\ell - \mathbf{f}_T)], \tag{4.12}$$

which can be substituted back into the composite velocity equation to yield

$$\nabla_{\mathbf{x}} \cdot \{\mathbf{v}_T + K[\Sigma_T' \nabla_{\mathbf{x}} \phi_T + \phi_T(\mathbf{f}_\ell - \mathbf{f}_T)]\} = 0. \tag{4.13}$$

For example, the case of a multicell spheroid as a growing poro-elastic medium can thus be written using the mass balance equation Eq. $(4.1)_1$, the momentum equation (4.11), the composite velocity equation (4.13), along with suitable reaction–diffusion nutrient equations from Eq. $(4.1)_2$.

This framework has been used by Ambrosi and Mollica,[12] Roose *et al.*[169] together with experimental data to show how tumour cell size is reduced by solid stress inside tumour spheroids. Ambrosi and Preziosi,[13] followed by Byrne and Preziosi,[50] modelled avascular tumour as a deformable porous medium in which the solid skeleton was assumed to be comprised of deformable cells bathed in

extracellular liquid containing nutrients, and included in the mass exchange between the solid and liquid constituents. It also included the viscous effects of cellular motion. This model was able to determine the stress distribution within the tumour, and how the stresses affected proliferation rates and tumour size.

In general, continuous models are able to exhibit the general behaviour of tumour growth, angiogenesis and invasion. However, all of these continuum approaches model average behaviour at a population level, and fail to examine phenomena that occur at the single cell level. This makes detailed modelling of processes such as angiogenesis difficult, as simply calculating average cell density fails to include the specific spatial structure of the vascular network. Furthermore, it is unclear if processes such as invasion and metastasis are driven by "average" population behaviour, or instead by cells which deviate from the mean. It is certainly possible that individual "rogue" cells drive the macroscopic processes of invasion or metastasis, and their behaviour would not be captured in a continuum model. In these cases, a discrete modelling approach must be taken in order to keep track of each individual cell.

4.2. *Discrete models*

Unlike continuum models, discrete models have the ability to track the behaviour of single cells. Due to biotechnological advances, there is an increasing amount of data available on phenomena at a single cell level which merit inclusion in mathematical models. These discrete approaches model single-cell scale phenomena and use upscaling techniques to examine the effect on macroscopic properties of the tumour.

Most discrete models utilise a combination of discrete cell-based models (such as cellular automata,[177,178,7,67] extended Potts,[189] random walk,[15,14] among others) to represent the behaviour of single cells, and continuous equations to model chemical gradients. Individual cells are then tracked on a lattice, where the cells of the lattice correspond to biological cells (as in cellular automata and random walk models) or each cell is made up of several lattice points (as in Potts models). The particular choice of lattice can be extremely important, and care must be taken to ensure that macroscopic properties are not driven by the specific structure of the lattice affecting cell movement. Also, the "neighbourhood" used to determine cell proliferation and movement (such as at which adjacent spaces a daughter cell can be placed) can affect the model behaviour. For example, on a 2-D rectangular lattice, a von Neumann neighbourhood includes the four spaces located north, south, east, and west of a given cell, while the Moore neighbourhood uses eight adjacent cells. Usually, a discrete model is comprised of a regular rectangular lattice for computational simplicity, however alternative geometries can be chosen. Furthermore, the lattice is usually fixed through time, but a free lattice can be constructed to move as a result of cell proliferation.

All of the main discrete models consider the state of each cell or populations of cells to be characterised by the vector variable $\mathbf{w} = \{\mathbf{x}, \mathbf{v}, \mathbf{u}\}$, where $\mathbf{x}$ is position,

v is velocity, and **u** is a vector detailing the cell's internal state, which might include information such as age, point in cell cycle, phenotypic characteristics, etc. This information would in turn affect the probability of a cell moving, proliferating, or changing state. In some cases, continuous models for cell density are discretised, usually using finite difference methods, providing transition rates for cells moving to an adjacent site. This can allow for the inclusion of stochastic effects. As in continuum models, assumptions regarding cell movement can be implemented, either by using phenomenological models such as diffusion or haptotaxis, or mechanical models. Any mechanical interactions would depend on the cell's position and velocity.

One advantage of this discrete method is the ease of embedding subcellular processes within each biological cell (which usually corresponds to an automaton cell). For example, the model can track each individual cell, and within each cell the simulation can run continuous mathematical models of metabolism or cell cycle. This potential for use as a multi-scale framework will be discussed in detail in Sec. 6.

In particular, a discrete approach is useful when modelling angiogenesis, as it allows modelling at the individual cell and vessel level. In this way, mathematical models can examine how endothelial cells link together and form functional blood vessels, and the precise structure of the vascular network. Anderson and Chaplain[15] began by simply examining endothelial sprout tips and the initiation of branching. Levine *et al.*[128,126] used reinforced random walks to examine angiogenic signalling, ECM degradation and subsequent migration of endothelial cells towards the tumour. Extensions of these models will be discussed further in Sec. 6.

Discrete models have also addressed the importance of visco-elastic effects and cell adhesion, with a focus on tumour invasion. Although the basics of tumour growth and movement into the surrounding tissue can be examined through continuum models (and in particular travelling wave solutions[176,133]), advances in imaging now allow us to visualise migration of individual cells and it has been suggested that perhaps single cell behaviour, in contrast to mean cell behaviour, might be driving invasion. Therefore, discrete modelling techniques are better suited to represent these aspects of tumour growth. In order to address these issues, Turner and Sherratt[189] used a discrete extended Potts model to investigate adhesion, proteolysis and haptotaxis using a thermodynamic approach of energy minimisation in a model of tumour invasion. This model was able to examine the relative importance of cell–cell adhesion and cell–matrix adhesion. Furthermore, recent work by Anderson *et al.*[16] utilises a hybrid discrete model incorporating cell-adhesion, cell-migration and phenotypic mutations, and suggests invasive "fingering" is driven by environmental heterogeneity. This model is described in detail in Sec. 6.

4.3. *From cellular to macroscopic models*

The various models reviewed in the preceding sections have been derived according to the classical approach of continuum mechanics, namely by using mass and momentum conservation equations properly closed by phenomenological models

corresponding to the material behaviour of the system. This approach is documented in the various chapters of the book,[146] containing lectures on the derivation of several models of biological tissues. Different models are obtained according to the different ways chosen to close the conservation and equilibrium equations.

On the other hand, the macroscopic behaviour should be properly related to the dynamics at the cellular level. In other words, macroscopic models should be derived from the underlying cellular models by suitable asymptotic methods developed by letting intercellular distances tend to those of the tissue level.

The above approach is widely studied in the case of classical particles by asymptotic methods developed in mathematical kinetic theory. In recent years, the analysis of the applicability of asymptotic methods has reached an important development in the so-called parabolic and hyperbolic limits or equivalently low and high field limits. The parabolic (low field) limit of kinetic equations leads to a drift–diffusion type system (or reaction–diffusion system) in which the diffusion processes dominate the behaviour of the solutions.

The specialised literature offers a number of recent contributions concerning various limits for parabolic diffusive models and the hyperbolic (high field) limit where the influence of the diffusion terms is of lower (or equal) order of magnitude in comparison with other convective or interaction terms. Therefore, different macroscopic models are obtained corresponding to different scaling assumptions. The literature in the field of asymptotic methods for classical particles is documented in the review papers by Villani,[191] Perthame,[161] Lachowicz[124] and Bonilla and Soler.[42]

The same methodological approach can be developed starting from multicellular models obtained by methods of generalised kinetic theory. Although the literature on this topic is not as vast as that for classical particles, a number of interesting results are available. On the other hand, several technical difficulties arise from the fact that particles are elements of inert matter, while cells are active particles belonging to living matter. In particular:

(i) The microscopic state of an active particle is characterised not only by position and velocity, but also by an additional microscopic state (we may call it *activity*) which represents biological function at a cellular level.
(ii) Microscopic interactions not only modify the microscopic state, but may also generate proliferative and/or destructive phenomena.

Technically, asymptotic methods amount to expanding the distribution function in terms of a small dimensionless parameter related to the intermolecular distances (the space-scale dimensionless parameter) that is equivalent to the connections between the biological constants. The limit that we obtain is singular and the convergence properties can be proved under suitable technical assumptions. In these papers biological systems are considered for which interactions do not follow classical mechanical rules, and biological activity may play a relevant role in determining the dynamics.

The paper by Othmer, Dunbar and Alt[151] is arguably the first one where this topic was addressed. Subsequent contributions in this area are due to various authors.[20–24,56,57,70,78,79,125,152,153] The above-cited papers deal with a variety of models of the kinetic theory of cellular systems corresponding to the modelling of cell interactions that are relevant in the biological system under consideration. The structure of the equations for tissue behaviour depends again on the predominance of one of the three aspects of the biological dynamics, i.e. encounter rate between cells, mutations, and proliferative/destructive events, with respect to the other two. Moreover, the structure of the mathematical equations modelling tissues may evolve in time due to the aforementioned dynamics. So far, the formal approach provides a range of structurally different macroscopic equations whose specific form depends on which of the above aspects of biological dynamics is assumed predominant.

Asymptotic methods have been applied to the following class of equations that model the evolution of the distribution function defined in Sec. 3.3. Specifically, mathematical results have been derived for models where the space dynamics is obtained by adding to the spatially homogeneous model a stochastic perturbation for the velocity.[152] Referring to the class of equations reviewed in Sec. 3.3, the model in absence of external action, can be written as follows:

$$\begin{aligned}\partial_t f(t,\mathbf{x},\mathbf{v},u) + \mathbf{v}\cdot\nabla_{\mathbf{x}} f_i(t,\mathbf{x},\mathbf{v},u)\\ = L[f](t,\mathbf{x},\mathbf{v},u) + C[f](t,\mathbf{x},\mathbf{v},u) + D[f](t,\mathbf{x},\mathbf{v},u),\end{aligned} \tag{4.14}$$

where $C[f]$ and $D[f]$ correspond, respectively, to conservative and proliferative/destructive interactions (in the absence of proliferation, due to genetic mutations, into a population different from that of the interacting cells). Moreover,

$$L[f] = \int_{D_{\mathbf{v}}} \Big[T(\mathbf{v},\mathbf{v}_*) f(t,\mathbf{x},\mathbf{v}_*,u) - T(\mathbf{v}_*,\mathbf{v}) f(t,\mathbf{x},\mathbf{v},u)\Big]\, d\mathbf{v}_* \tag{4.15}$$

models a linear velocity-jump process, where ν is the turning rate or turning frequency (hence $\tau = \frac{1}{\nu}$ is the mean run time) and $T(\mathbf{v},\mathbf{v}_*)$ is the probability kernel for the new velocity $\mathbf{v} \in D_{\mathbf{v}}$ assuming that the previous velocity was $\mathbf{v}_*$. This corresponds to the assumption that cells choose any direction with bounded velocity. Specifically, the set of possible velocities is denoted by $D_{\mathbf{v}}$, where $D_{\mathbf{v}} \subset \mathbb{R}^3$, and it is assumed that $D_{\mathbf{v}}$ is bounded and spherically symmetric (i.e. $\mathbf{v} \in D_{\mathbf{v}} \Rightarrow -\mathbf{v} \in D_{\mathbf{v}}$). Further, notation $d\mathbf{v}$ stands for integration over three-dimensional velocity space.

Let us first consider the hyperbolic scaling corresponding to:

$$t \to \varepsilon\, t, \quad \mathbf{x} \to \varepsilon\, \mathbf{x} \quad \Rightarrow \quad t\,\nu = \frac{1}{\varepsilon}, \tag{4.16}$$

and introduce the parameters

$$\nu = \frac{1}{\varepsilon}, \quad \eta = \varepsilon^{q-1}, \quad \mu = \varepsilon^{\delta}, \quad q \geq 1, \quad \delta \geq 0. \tag{4.17}$$

Therefore, the scaled non-dimensional model takes the form:

$$(\partial_t + \mathbf{v}\cdot\nabla_{\mathbf{x}}) f_\varepsilon = \frac{1}{\varepsilon}(\mathcal{L}(f_\varepsilon) + \varepsilon^q \mathcal{C}(f_\varepsilon, f_\varepsilon) + \varepsilon^{q+\delta}\mathcal{D}(f_\varepsilon, f_\varepsilon)). \tag{4.18}$$

The approaches in the literature are limited to the above class of models, while the case of proliferation related to genetic mutations has not yet been addressed. The derivation needs the following assumptions:

Assumption 4.1. (Solvability conditions) The turning operator $\mathcal{L}$ satisfies, in the whole physical space, the following solvability conditions:

$$\int_{D_{\mathbf{v}}} \mathcal{L}(f)(\mathbf{v})\, d\mathbf{v} = 0, \qquad \int_{D_{\mathbf{v}}} \mathbf{v}\mathcal{L}(f)(\mathbf{v})\, d\mathbf{v} = \mathbf{0}, \tag{4.19}$$

where $\mathcal{L}(f)$ is the linear operator, corresponding to (4.15), acting on f (the arguments t and $\mathbf{x}$ have been dropped to simplify notation), and integration is over the whole velocity space.

Assumption 4.2. (Kernel of $\mathcal{L}$) There exists a unique function $M_{\rho,U} \in L^1(D_{\mathbf{v}}, (1+|\mathbf{v}|)\, d\mathbf{v})$, for all $\rho \in [0, +\infty)$ and $U \in \mathbb{R}^n$, such that

$$\mathcal{L}(M_{\rho,U}) = 0, \quad \int_{D_{\mathbf{v}}} M_{\rho,U}(\mathbf{v})\, d\mathbf{v} = \rho, \quad \int_{D_{\mathbf{v}}} \mathbf{v}\, M_{\rho,U}(\mathbf{v})\, d\mathbf{v} = \rho\, U,$$

where ρ is the density and U is the mass velocity.

Let us now consider the equilibrium distribution given in the form $f_0 = M_{\rho,U}$ and look for the solution f_ε as a perturbation of this equilibrium in the following way:

$$f_\varepsilon(t, x, v, u) = M_{\rho,U} + \varepsilon\, g(t, \mathbf{x}, \mathbf{v}, u). \tag{4.20}$$

The result of the paper[24] shows that hyperbolic equations with different source terms are obtained as follows:

Case 4.1. $\delta \geq 0$, and $q > 1$: First order moments with respect to ε generate the hyperbolic system without source term:

$$\begin{cases} \partial_t \rho + \nabla_{\mathbf{x}}(\rho U) = 0, \\ \partial_t(\rho U) + \nabla_{\mathbf{x}}(\rho U \otimes U + p) = 0. \end{cases} \tag{4.21}$$

This corresponds to negligible biological (both conservative and proliferative/destructive) activities.

Case 4.2. $\delta \neq 0$, and $q = 1$: In this case, in first order with respect to ε, the following hyperbolic system with a source term related to conservative interactions is obtained:

$$\begin{cases} \partial_t \rho + \nabla_{\mathbf{x}}(\rho U) = \displaystyle\int_{D_{\mathbf{v}}} \mathcal{G}(M_{\rho,U}, M_{\rho,U})(t, \mathbf{x}, \mathbf{v})\, d\mathbf{v}, \\ \partial_t(\rho U) + \nabla_{\mathbf{x}}(\rho U \otimes U + p) = \displaystyle\int_{D_{\mathbf{v}}} \mathbf{v}\, \mathcal{G}(M_{\rho,U}, M_{\rho,U})(t, \mathbf{x}, \mathbf{v})\, d\mathbf{v}. \end{cases} \tag{4.22}$$

Case 4.3. $\delta = 0$ and $q = 1$. In this case, in first order with respect to ε, the following hyperbolic system with a source term related to both conservative and proliferating interactions is obtained:

$$\begin{cases} \partial_t \rho + \nabla_{\mathbf{x}}(\rho U) = \displaystyle\int_{D_{\mathbf{v}}} \mathcal{G}(M_{\rho,U}, M_{\rho,U})(t,\mathbf{x},\mathbf{v},u)\, d\mathbf{v} \\ \qquad\qquad + \displaystyle\int_{D_{\mathbf{v}}} \mathcal{I}(M_{\rho,U}, M_{\rho,U})(t,\mathbf{x},\mathbf{v},u)\, d\mathbf{v}, \\ \partial_t(\rho U) + \nabla_{\mathbf{x}}(\rho U \otimes U + p) = \displaystyle\int_{D_{\mathbf{v}}} \mathbf{v}\mathcal{G}(M_{\rho,U}, M_{\rho,U})(t,\mathbf{x},\mathbf{v})\, d\mathbf{v} \\ \qquad\qquad + \displaystyle\int_{D_{\mathbf{v}}} \mathbf{v}\,\mathcal{I}(M_{\rho,U}, M_{\rho,U})(t,\mathbf{x},\mathbf{v})\, d\mathbf{v}. \end{cases} \tag{4.23}$$

It is worth remarking that the influence of the turning operator $\mathcal{L}$ on the macroscopic equation only comes into play through the equilibrium state $M_{\rho,U}$ in the computation of the right-hand side and the pressure tensor.

A different scaling leads to *diffusive models*. Specifically, consider the following:

$$\eta = \varepsilon^q, \quad \mu = \varepsilon^\delta, \quad q, \delta \geq 0 \quad \text{and} \quad \nu = \frac{1}{\varepsilon^p}, \quad p > 0, \tag{4.24}$$

where ε is a small parameter which will be allowed to tend to zero. In addition, the slow time scale $\tau = \varepsilon t$ is used so that the following scaled equation is obtained:

$$\varepsilon \partial_t f_\varepsilon + \mathbf{v} \cdot \nabla_{\mathbf{x}} f_\varepsilon = \frac{1}{\varepsilon^p}\, \mathcal{L} f_\varepsilon + \varepsilon^q g(f_\varepsilon, f_\varepsilon) + \varepsilon^{q+\delta} I(f_\varepsilon, f_\varepsilon). \tag{4.25}$$

The following assumption is needed:

Assumption 4.3. (Velocity distribution $M(\mathbf{v})$) There exists a bounded velocity distribution $M(\mathbf{v})$, independent of t and $\mathbf{x}$, such that the detailed balance $T(\mathbf{v}^*, \mathbf{v})M(\mathbf{v}) = T(\mathbf{v}, \mathbf{v}^*)M(\mathbf{v}^*)$ holds with normalised flow:

$$\int_{D_{\mathbf{v}}} M(\mathbf{v})\, d\mathbf{v} = 1, \qquad \int_{D_{\mathbf{v}}} \mathbf{v}\, M(\mathbf{v})\, d\mathbf{v} = \mathbf{0}.$$

Moreover, the kernel $T(\mathbf{v}, \mathbf{v}^*)$ is bounded and such that:

$$T(\mathbf{v}, \mathbf{v}^*) \geq \sigma\, M, \quad \forall\, \mathbf{v}, \mathbf{v}^* \in D_{\mathbf{v}} \times D_{\mathbf{v}},$$

for all times and in the whole spatial domain.

Let us now consider the local density $n_\varepsilon(t, \mathbf{x})$ defined as follows:

$$n_\varepsilon(t,\mathbf{x}) = \int_{D_{\mathbf{v}} \times R} f_\varepsilon(t,\mathbf{x},\mathbf{v},u)\, d\mathbf{v}\, du$$

and the density $n(t, \mathbf{x})$ given by

$$n(t,\mathbf{x}) = \int_{D_{\mathbf{v}} \times R} f(t,\mathbf{x},\mathbf{v},u)\, d\mathbf{v}\, du\,.$$

It can be proved[20] that the density n_ε converges weakly to n which is a solution of (4.14).

Case 4.4. $q = 1$, $\delta \neq 0$ or $q > 1$, $\delta \geq 0$

$$p = 1: \quad \partial_t n - \nabla_{\mathbf{x}} \cdot (D \cdot \nabla_{\mathbf{x}} n) = 0 \tag{4.26}$$

and

$$p > 1: \quad \partial_t n = 0, \tag{4.27}$$

where D is the diffusion coefficient.

Case 4.5. $q = 1$, $\delta = 0$

$$p = 1: \quad \partial_t n - \nabla_{\mathbf{x}} \cdot (D \cdot \nabla_{\mathbf{x}} n) = \langle M^2 \rangle_{\mathbf{v}} n^2$$

and

$$p > 1: \quad \partial_t n = \langle M^2 \rangle_{\mathbf{v}} n^2, \tag{4.28}$$

where $\langle \cdot \rangle_{\mathbf{v}}$ denotes average of the quantity $\cdot$ over velocity. Additional examples and various applications are reported in Refs. 20, 21 and 23.

4.4. *Moving boundary models*

In the above, we have shown how the macroscopic description of biological tissues can be obtained either by the classical approach of continuum mechanics or, maybe more appropriately, by suitable hydrodynamic limits applied to the underlying microscopic description at the cellular level. Moreover, it has been shown that different mathematical models, namely parabolic, hyperbolic, or simply evolution equations, can be obtained corresponding either to different closures of the conservation equations in the continuum mechanical approach, or to different ratios between the mechanical and biological timescales.

One of the most relevant applications of continuum models is the study of moving boundary problems wherein it is assumed that growth of solid tumours occurs in an environment where nutrients nurture their development in the face of chemical factors that inhibit growth, while other agents, for example, the immune system and macrophages, are also involved in growth arrest.

The above approach is documented in several papers.[36,37,59,63,86,85,58,131,129] The essential concepts were first reported in the pioneering papers by Greenspan[99] and Adam.[2,54,4] Further references will be given in Sec. 7 in connection with the review of some challenging mathematical problems generated by the study of moving boundary problems.

This subsection presents various aspects of the methodological approach. We refer the reader to the paper by De Angelis and Preziosi[63] for full technical details. In particular, we focus on some critical conceptual difficulties that arise. Some of these have already been addressed, as will be described in Sec. 6.

The biological system is represented in Figures 2 and 3 schematically:

$\mathcal{T}$ is the inner zone of the solid tumour;
$\mathcal{D}$ is the domain of the outer environment;
$\partial\mathcal{T}$ is the boundary separating $\mathcal{T}$ from $\mathcal{D}$.

To make the model tractable, the geometry is simplified. For example, in the simplest cases $\mathcal{T}$ is assumed to be spherical, cylindrical or even one-dimensional.

The second step consists of identifying the macroscopic variables that can possibly describe the state of the overall system viewed as a continuum and then developing a strategy to reduce the complexity related to the large number of components to a limited number of variables. For instance, still referring to the above cited paper,[63] the following variables are selected:

— the density u_1 of living tumour cells;
— the density u_2 of necrotic tumour cells;
— the concentration u_3 of the factor that inhibits tumour growth;
— the concentration u_4 of the factor that activates angiogenesis;
— the density u_5 of endothelial cells;
— the concentration u_6 of nutrient.

The above variables represent an overall system which is much more complex with a greater number of components. Reduction is achieved by grouping in each variable the collective behaviour of several cooperative actions. Specifically, the variables u_1 and u_2 are defined in $\mathcal{T}$, while u_3, u_4, u_5 and u_6 are defined in the whole domain $\mathcal{T} \cup \mathcal{D}$.

The mathematical models in the domains $\mathcal{T}$ and $\mathcal{D}$ consist of systems of PDEs derived according to the modelling of the following, briefly described, biological phenomena:

Mitosis occurs only if tumour cells receive a sufficient quantity of nutrient, greater than that needed for survival. On the other hand, division can be inhibited by mitotic inhibitors. When the nutrient levels are too low, cells undergo necrosis.

Movement of cells occurs towards zones of lower cell density, while necrotic tumour cells do not move and naturally disintegrate.

Endothelial cells proliferate with a rate dependent on the chemical factors emitted by tumour cells to activate angiogenesis. Capillary sprouts further facilitate the diffusion of nutrients.

Proteins, named angiostatins, have the ability to reduce the proliferation of endothelial cells and hence reduce angiogenesis.

The aforementioned system of PDEs is coupled on the free boundary $\partial\Omega$ by suitable compatibility conditions generally described by ordinary differential equations.

The paper[63] describes in detail how the above biological considerations are converted into a specific mathematical model. Several papers, such as those cited

in this subsection, report technical variations corresponding to different biological situations, while mathematical problems generated by the applications of models to the analysis of tumour progression are reviewed in Sec. 7 below.

5. From Genetic Mutations to Onset of Cancer Phenotype

As we have mentioned, to implement modelling at the cellular scale, we require information on cell function that arises from the dynamics at the intracellular (molecular) level. This information is also necessary for models at the macroscopic scale because the whole system is driven, to some extent, by genetic mutations. (Of course, this is a simplified view as it ignores feed-back down the spatial scales.) This section deals with modelling approaches at the microscopic (genetic) scale focused to extract, from the lower scale, the above-mentioned inputs to the higher scales.

The paper by Vogelstein and Kinzler[192] is an essential reference to understand how the over-expression and under-expression of certain genes can generate the anomalous behaviour of cells leading to the onset of neoplasia followed by clonal expansion. Quoting the above-cited paper

Alterations in three types of genes are responsible for tumourigenesis: *oncogenes*, *tumour-suppressor genes*, *and stability genes.*

Oncogenes are mutated in ways that render the gene constitutively active or active under conditions in which the wild-type gene is not. Oncogene activations can result from chromosomal translocations, *from gene amplifications or from subtle intragenic mutations affecting crucial residues that regulate the activity of the gene product.*

Tumour-suppressor genes are targeted in the opposite way by genetic alterations: *mutations reduce the activity of the gene product. Such inactivations arise from missense mutations at residues that are essential for its activity*, *from mutations that result in a truncated protein*, *from deletions or insertions of various sizes*, *or from epigenetic silencing.*

A third class of cancer genes, *called stability genes or caretakers*, *promotes tumorigenesis in a completely different way when mutated. This class includes mismatch repair*, *nucleotide-excision repair and base-excision repair genes responsible for repairing subtle mistakes made during normal DNA replication or induced by exposure to mutagens.*

According to the above literature, it is well established that the onset of cancer is caused by under- or over-expression of those genes which are responsible for tumourigenesis. This can occur during DNA replication, when a cell does not have sufficient ability to repair DNA corruption. It can also be caused by interaction with other genes, or with the external environment. Stochastic events are typical in the phenomena under consideration.[97]

The molecular biology literature on the above topic is vast. The interested reader is referred to the review paper by Vogelstein and Kinzler,[192] or to the book by

Weinberg.[197] The review by Baylin and Ohm[19] reports on the role of gene activation and silencing in carcinogenesis, while the role of genetic therapies is reported in the paper by Mezhir *et al.*[142] (see also the review by Futreal *et al.*[89]). While large strides have been made biologically, the mathematical approaches are still at a preliminary stage.

Essentially, the mathematical literature has been devoted to modelling gene interactions and their evolution. Although a systematic approach is not yet available, some interesting results have already appeared. Two mathematical approaches are examined below: the first one is due to Komarova,[120–122] while the second one is by Gatenby *et al.*,[95,96] both dealing with different aspects of the problem (see also Tiuryn, Wójtowicz and Rudnicki[188] and Michor, Iwasa and Nowak[145]). An important reference is the recently published book by Frank.[84]

Komarova's approach is focused on the stochastic dynamics of gene interaction in cancer initiation and progression related to mutations which generate loss and gain of function. She shows how cumulative mutations can lead to tumour progression.

An interesting aspect of this approach is the development of some preliminary ideas to relate some of the relevant functions of cells to the dynamics of genes. The next step is to develop Komarova's ideas to look for the link between the molecular and cellular scales. The basic idea consists of developing a stochastic game theory[93] as the essential tool to model the output of gene interaction. Technical developments based on a deeper insight into stochastic dynamics of gene interactions are presented in the paper by Tiuryn, Wójtowicz and Rudnicki.[188]

Relatively simpler models assume that cancer mutations can be described by a deterministic description using ordinary differential equations.[180] A deeper insight into the dynamics at the molecular level is necessary to capture the essence of the complexity of the system viewed as an evolutionary and ecological process.[140]

In general, the derivation of a model suitable for describing the time evolution of gene activation and silencing, due to gene interaction and action from the micro-environment, remains a challenging and fascinating open problem. A successful approach should also lead to incorporating the subsequent influence on biological functions expressed by cells as the natural input to models developed at the cellular scale.

The mathematical approach proposed by Gatenby[94–96,174,175] is essentially based on the idea that the onset of tumourigenesis is an evolutionary process where cells follow a Darwinian interaction of altered cellular genotypes with changing micro-environment.[16] Gatenby, Vincent and Gilies[96] propose a population competition model where the "*winners*" are determined by their phenotypic fitness relative to other populations in the environment. Winners proliferate at the expense of losers, while phenotypic properties are retained or lost depending on their contribution to individual fitness. The model consists of a system of ordinary differential equations where gain and loss terms are phenomenologically modelled according to the above interaction dynamics.

Various authors have proposed that evolutionary processes play a crucial role in biological modifications at the cellular scale with a timescale much shorter than the one needed for mutations at the species level. Nowak and Sigmund[149] state:

evolutionary game theory is an essential component of a mathematical and computational approach to biology.

The above phenomenological description and theoretical approaches motivate physicists to look at the use of generalisations of the classical methods of statistical and quantum mechanics. The conjecture proposed in paper[29] suggests developing at the molecular scale some ideas already exploited at the cellular scale. In other words, the conjecture is to use structures (3.7)–(3.11) to describe the dynamics at the molecular scale and derive, out of this dynamics, the above main cellular interaction terms.

In this case, the internal microscopic variable is gene expression, the overall state of the system is described by the distribution function over this state, while the gain and loss terms should refer both to interactions between genes and with the external environment. Onset of instability, referred to as oncogenesis, then leads to carcinogenesis.

6. Complexity Analysis and Multiscale Modelling

As already mentioned several times, tumourigenesis is a multiscale phenomenon. Figure 4 is an attempt to capture, in a schematic representation, the essence of the multiscale nature of the system under consideration, illustrating that cellular and molecular events continue to play a crucial role in the temporal evolution of the tumour throughout its development.

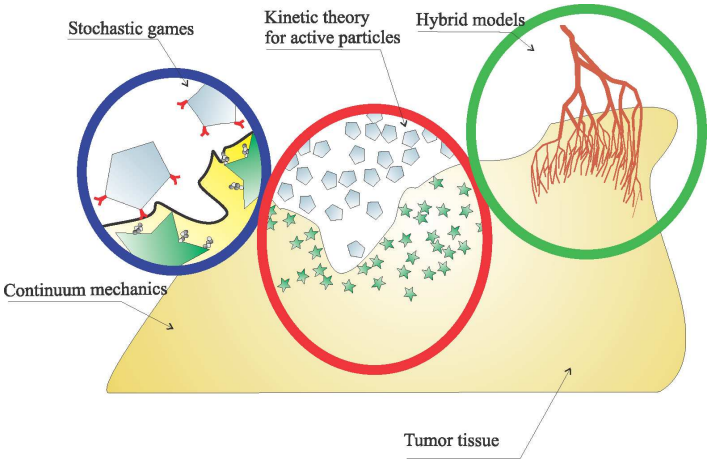

Fig. 4. Schematic multiscale representation of tumour growth: gene interactions (stochastic games), cells (kinetic theory), tissues (continuum mechanics), mixed (hybrid models).

Multiscale analysis involves various aspects of the modelling approach. In particular, mathematical models at a certain scale need to be consistent with the lower and higher scales. In other words, models at the tissue level (macroscopic) have to be obtained by suitable asymptotic methods from the underlying cellular (microscopic) scale, while parameters of models at the cellular scale should be identified from the lower molecular scale. Moreover, the overall system can be regarded as a network of several interacting subsystems, each developed at a specific scale, while interactions between contiguous systems need to deal with compatibility conditions (in some cases boundary conditions) at each specific scale. The above issues will be analysed later after an overview on the existing literature.

Although a systematic development of multiscale approaches is very recent, some papers already anticipate the need for dealing with a modelling approach suitable to account for different scales. For instance, the role of cellular dynamics on the rough surface of solid tumours is studied in Refs. 45 and 46. Experimental evidence shows that the dynamics at the cellular scale can play a relevant role on the evolution of solid tumours (continuum mechanics). The paper by Levine, Sleeman and Nilsen-Hamilton[127] includes within a macroscopic approach a network of several phenomena at the cellular level such as cellular penetration and formation of capillary sprouts. Random walk methods[141] are introduced to deal with two scales of diffusion. The role of cell adhesion (cellular scale) on angiogenesis analysed at the macroscopic scale is dealt with in Refs. 15 and 139. The coupling of models at the cellular scale to identify the tissue properties of models at the macroscopic scale is studied by Marchiniak-Cozchra and Kimmel[134,136,139] in an analysis of the early stages of tumour growth.

One of the first papers to introduce a systematic approach to a multiscale modelling of the overall system viewed as a system of systems is arguably due to Alarcon, Byrne and Maini[7] for vascular tumours. This initial model has been extended considerably in a subsequent series of papers.[9,49,10] The focus of these papers is on modelling vascular tumour growth, incorporating subcellular cell cycle processes, cellular interactions, macroscopic properties of the vascular network and invasive patterning.

In their earlier paper,[7] Alarcon, Byrne and Maini used a cellular automaton to model the effect of vascular dynamics on competition between normal and tumour cells. They formulated a model of vascular adaptation based on hydrodynamic principles and known features of vascular adaptation, such as change in vessel radius. The model also included elements of blood rheology and haematocrit and incorporated the dependence of blood viscosity on haematocrit and vessel radius. At each time step of the cellular automaton, the oxygen distribution and vascular network properties were calculated, and then the states of the normal and tumour cells (such as quiescent, proliferative, etc.) were determined based on local oxygen levels.

In subsequent papers, subcellular cell-cycle models were included, such that inside each cell was a set of ODEs representing the progression through cell-cycle phases due to nutrient availability and kinease activity.[8] In this way, they

formulated a multiscale model integrating the vascular, cellular and intracellular levels: the vascular network responds to chemical stimuli produced at the cellular level and the nutrient distribution provided by the vasculature affects cellular (apoptosis, quiescence, etc) and subcellular (production of VEGF, expression of p53) processes. With each subsequent paper, they have been able to incorporate an increasingly complex level of interaction and feedback between the various scales. Figure 5, from the paper by Betteridge *et al.*,[38] details one example of this type of interacting network between the various spatial scales.

Very few multiscale models have attempted to include genetic mutations. Smallbone *et al.*[175] examined the development of ductal carcinoma *in situ*, and how the micro-environment could effect the somatic evolution of cancer cells towards glycolytic and acid-resistant phenotypes. Their model allowed reversible heritable mutations which affect survival and proliferation (such as the ability to grow away from the basement membrane, the ability to upregulate glycolysis in hypoxic environments, and the ability to survive acidic regions). This model has made experimentally verifiable predictions of nodular growth of constitutively upregulated glycolytic populations.[94]

Another notable multiscale model using phenotypic mutations is due to Anderson *et al.*[16] They also use a hybrid model, examining the interaction between cellular and micro-environmental factors. Their model links the environment and nutrient availability to phenotypic mutations which alter a single cell's phenotypic traits related to proliferation, cell–cell adhesion, oxygen consumption, movement, etc. PDEs are used to represent the oxygen, matrix degradative enzymes and ECM. Within this model, cells are allowed to evolve and change their phenotype through

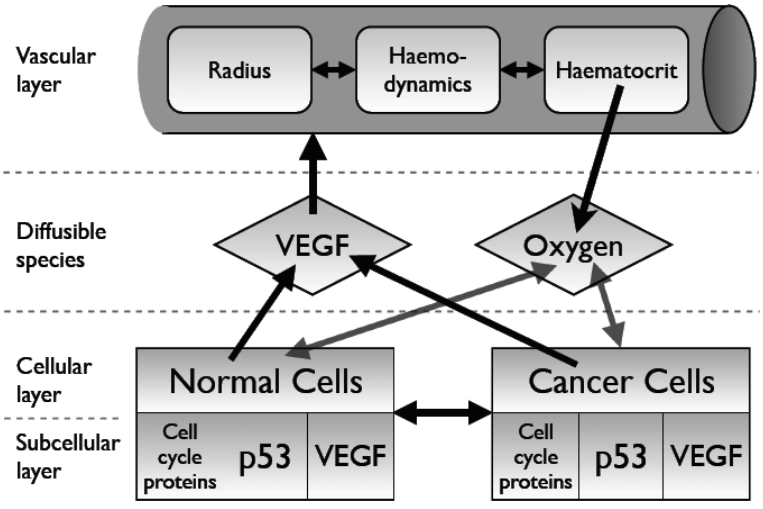

Fig. 5. Multiscale interactions of vascular tumour growth (from Betteridge *et al.*[39]). See text (and the original paper) for fuller details.

heritable mutations gained or lost during proliferation. Two different mutation schemes were used in the simulations, a linear scheme where the cells could mutate to more and more aggressive phenotypes, and another random scheme where they could "jump" randomly between 100 pre-selected phenotypes, ignoring the parent phenotype. Interestingly, both mutation schemes yielded similar results, one where the tumour evolves towards an increasingly aggressive phenotype, with higher proliferation, lower cell–cell adhesion and higher matrix degradation.

The main focus of the Anderson *et al.* paper,[16] however, was the importance of the environment in tumour progression. By varying the environmental conditions (such as manipulating oxygen concentration throughout the simulation or changing the underlying matrix) the model predicted that a harsh tumour environment exerts a selective force which results in an invasive, fingering tumour which is dominated by a few clones of the most aggressive phenotype. However, homogeneous, well-oxygenated environments produce more rounded tumours with less fingering infiltration and selection of less aggressive phenotypes. The implications for tumour treatment are clear: treatments which provide a hostile environment in order to kill the tumour may, in fact, be creating a strong selection pressure towards more aggressive, more invasive tumours.

An alternate multiscale modelling framework has been developed by Kim, Stolarska and Othmer[116] in a recently published paper on *in vitro* tumour spheroid growth. The previous approaches discussed[16,7–9] have both used lattice-based discrete models for the cells in the simulation. However, this quickly presents a serious computational problem, as even a tumour spheroid with a diameter of 2 mm contains approximately 2×10^6 cells. Multiscale models wherein each of these cells also contains a system of ODEs modelling the cell cycle, and each of the cells mechanically interacts with each other, very quickly become computationally intractable. To address this computational problem, Kim *et al.*[116] formulated a model which only treats the rapidly proliferating rim region as discrete, while using a continuum model to describe the remaining quiescent and necrotic regions, as well as the surrounding gel or matrix. Their reasoning is that only the mechanical properties of the quiescent, necrotic and gel regions are important to spheroid growth, hence these regions can simply be described by continuum equations. Therefore, there are four regions in the model: the necrotic core, the quiescent zone, the proliferating rim and the surrounding gel or matrix. The continuum gel, quiescent, and necrotic regions are assumed to be homogeneous materials, but with different mechanical properties. In the proliferating zone, variations in cell-cycle time, cell-size, metabolic state, mobility, etc. are all crucial to tumour formation and therefore are described by a discrete (individual-based) model. This novel hybrid model can still address single cell–cell adhesion and invasive patterning in the proliferative zone, while simplifying computationally the overall system, as the proliferating zone is only comprised of a few hundred cells.

The forces on each particular cell in their model consist of the active force exerted on neighbouring cells or substrate, the reactive force due to forces exerted

from other cells on it, the static frictional force due to cell–cell or cell–matrix adhesion, and the dynamic drag forces arising from breaking adhesive bonds with neighbouring cells during movement. Individual proliferating cells are treated as oriented ellipses which can grow and deform due to external forces. The cytoplasm is modelled as an incompressible viscoelastic solid and the continuum regions are treated as linear viscoelastic materials with different material properties.

The model is able to exhibit some experimentally observed behaviours, such as the existence of a constant thickness proliferating rim (of about 100 microns), independent of the stiffness of the gel. Furthermore, it accurately predicts that gel stiffness affects tumour growth rate. Although in a preliminary stage, this approach provides an excellent framework for a computationally tractable model of tumour growth which includes mechanical forces and intracellular dynamics. The computational grid in the case of avascular tumour is represented in Fig. 6, from the paper by Kim, Stolarska and Othmer.[116]

Other authors have followed a multiscale approach to model the overall system as a network of several interacting subsystems corresponding to different biological situations, e.g. avascular tumours.[111,116] It is well known that applied mathematicians are strongly attracted by the above approach, so that it is expected that many variations on this theme will soon appear. A critique on the selection of mathematical models to be used at each scale can be found in the survey paper.[26]

The above-cited papers[9,49,10] have the merit of having defined the methodological aspects of multiscale modelling, where the overall system can be viewed as a system of interacting subsystems, each of which is localised in a well-defined

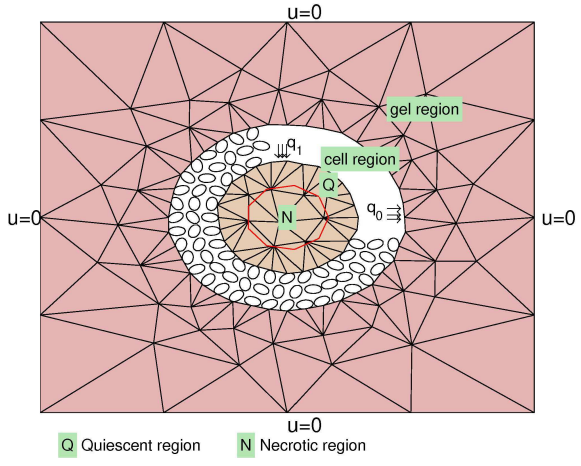

Fig. 6. Computational grid for tumour spheroid growth, with a continuum necrotic core (N), quiescent region (Q) and surrounding gel, along with a discrete proliferating cell region (white). Here $\mathbf{q}_0$ is the boundary force between the gel and proliferating regions, $\mathbf{q}_1$ is the boundary force between the proliferating and quiescent regions, and $\mathbf{u}$ is the displacement field.[116]

domain. In some cases, two (or more) different systems occupy the same domain. As can be seen, the above approach divides the modelling into four sub-processes:

6.1 The decomposition of the whole system into subsystems should be related to the theory of modules,[103] so that each subsystem is related to a well-defined main biological function.

6.2 Each subsystem (module) has to be localised in a well-defined domain D_i, in contact with other domains within the overall domain D, where $D = \cup_{i=1}^{m} D_i$. The number of subsystems may be greater than the number of domains m as more than one subsystem can be localised in the same domain.

6.3 A model of each subsystem can be derived at one of the three scales described in Sec. 2. The space variable can, for some specific models, be characterised by discrete directions as visualised on the right-hand side of Fig. 4. Models of each subsystem may be developed, as documented in Ref. 7, by using a hybrid approach.[67]

6.4 The overall implementation of the model needs to be completed by modelling interactions of subsystems within the same domain and by compatibility conditions, in some cases to be regarded as boundary conditions, between models located in two contiguous domains.

Some speculations are developed in what follows for each of the above issues. The discussion is motivated both by the existing literature in the field and by the various examples reported in the preceding sections.

6.1. *Decomposition into subsystems as modules*

The complexity of the system is such that all the components cannot be included if we wish to develop practical models. One possible approach to deal with this "curse of dimensionality" is the theory of modules proposed by Hartwell *et al.*,[103] wherein each subsystem is regarded as a module, according to the specific biological functions expressed by the cooperative action of various entities.

It is interesting to observe that the identification of the aforementioned functions may depend, at least in some cases, on the particular phenomena that are under study. Consequently, the decomposition of the system into modules is not unique, but depends on the biological phenomena that are being analysed. The modular approach is motivated in the brief note by Herrero,[104] while it is related to Hartwell *et al.*'s theory in various papers dealing with models at the cellular level.[28,29]

6.2. *Localisation of modules*

Localisation of modules is a necessary step for the statement of mathematical problems. Two specific cases generally appear:

(i) Each module M_i occupies a well defined domain D_i, so that the number m of modules is equal to the number of domains corresponding to the decomposition of the whole domain;

(ii) More than one module, say M_i^h and M_i^k, is localised in the domain D_i. Therefore, the number of modules is greater than the number of domains.

The mathematical models corresponding to each domain-module are generally derived at a specific scale in each domain.

6.3. *Scaling and hybrid systems*

As already mentioned, models at different scales correspond to different modules. For instance, the assembly of the entire model (in some cases, a network) can be constituted by models at the molecular scale, interacting with models at the cellular or macroscopic scale. Similarly, models at the cellular scale may interact with models at the macroscopic scale.

While a systematic approach to scaling does not yet exist in the literature, there are some particular applications that provide a useful background for its development. Some specific examples are briefly described below and are critically analysed in view of the statement of key mathematical problems.

6.4. *Compatibility and boundary conditions*

The well-posedness of mathematical problems for a system constituted by several interacting modules requires the necessary initial and boundary conditions for each model related to each module. This aspect has been dealt with in the various applications reviewed above. The analysis of the difficulty of the problem and some suggestions are now proposed in a general context.

6.4.1. *Compatibility conditions on boundaries*

The statement of boundary conditions on the surface of separation between two contiguous domains needs to be related to the compatibility conditions that describe the state in each domain. In some cases the relation may be nonlinear. One approach to this problem has been addressed by Kim, Stolarska and Othmer *et al.*,[116] who consider compatibility conditions between the continuum tissue, discrete proliferating region, continuum quiescent and necrotic regions as shown in Fig. 3.

The variables describing the states of the systems may refer to different scales, for instance macroscopic and cellular. Therefore, compatibility conditions between boundaries may need to transfer the variable from one scale to the other. Specifically, macroscopic information from the cellular scale is simply obtained by moments, so that compatibility conditions refer to functions of moments. This approach is sufficient going from the cellular to the macroscopic scales, while it is not so for the reverse direction.

6.4.2. *Compatibility conditions inside a module*

Alternatively, variables describing different scales may exist within the same module. These variables may need compatibility conditions which are different from

those needed on the boundaries of the model, or between modules as described above. Instead, compatibility conditions between scales within a single module may involve taking asymptotic limits and finding matching conditions. For example, a model of angiogenesis might have different equations describing the vascular network at each scale (such as at the capillary, grid and tissue levels). Therefore, the equations which describe single capillaries would need to have matching conditions at the asymptotic limit to the equations describing those of the capillary network. Again, in this case this approach is sufficient when moving "up" scale levels, such that the asymptotic limit of the smaller scale matches the equation at the higher scale.

6.5. *Critical analysis*

To develop a workable multiscale modelling framework, a number of challenges must be overcome. These include:

• *Analysing large interacting systems.* One consequence of incorporating numerous signalling pathways or interacting cytokines is a significant increase in system size and complexity. Mathematicians need to develop ways of analysing large interacting systems without sacrificing the amount of information that can be gained. How much can one actually conclude from a huge system of equations with numerous variables and parameters? What are the best ways to analyse these systems, and is the added complexity actually helping us learn anything? What methods are used to judge how good these models are at representing the system? Importantly, none of the models previously mentioned truly model subcellular pathways from genetic alterations to phenotypic changes. Although much of this is not yet known biologically, techniques for accurately modelling these large interacting systems with computationally tractable models need to be developed.

• *Justification of coupling/decoupling.* It is still unclear when it is appropriate to decouple systems which are intrinsically coupled. For example, the traditional form of Fickian diffusion decouples the chemistry from the mechanics of the system. Future mathematicians need to develop methods to examine under what conditions it is suitable to decouple an otherwise coupled system.

• *Mechanistic models.* Current research has failed to provide truly mechanistic models of angiogenesis and metastasis. In the case of angiogenesis, there is a lack of biologically detailed modelling of branching, anastomosis, vascular normalisation and the "brush border effect". Recent work by McDougall, Anderson and Chaplain[138] has attempted to address this problem by making branching at capillary tips dependent on wall shear stress as well as chemical gradients. Also, theoreticians have completely ignored the processes of active cell migration to blood vessels, intravasation, extravasation, and distant site colonisation in metastatic spread.

• *Cell geometry.* Few models account for changes in cell shape and deformation, which is known to affect cell-cyle regulation, growth, proliferation and movement.

• *Modelling transport properly.* Future modellers will have to consider the appropriate way to model diffusion in these systems (if, indeed, diffusion is the correct way to model transport). For example, what is the appropriate way to model complex nonlinear diffusion? Mathematicians dealing with porous media have utilised anomalous (or fractional) diffusion to describe diffusion through heterogeneous media (Metzler and Klafter[141]). Future modellers should examine the various possible forms for diffusion terms. How does diffusion depend on mechanics? How do stresses and strains affect the diffusive process?

Modelling chemotaxis and haptotaxis properly. As with diffusion, alternate forms of chemotaxis and haptotaxis should be studied. For example, how can biphasic haptotaxis be properly modelled? Othmer and Stevens[153] investigated various mathematical forms for the chemotaxis term at the macroscopic (tissue) level, based on behaviour at the individual cell level. They examined the relative importance of short versus long range signalling, and if the manner of detection and transduction of the chemotactic signal affected cell movement. Further work such as this will help clarify our understanding of what types of mathematical terms should be used in different biological situations.

• *Modelling invasion as an active, coordinated process.* Future models will need to address the growing body of literature indicating that invasion is an active process involving various forms of cell motility and that metastasis to distant sites involves coordination and signalling between different cell types (Friedl and Wolf,[88] Kaplan *et al.*[123]).

• *Calculation of error.* Although there are well established techniques for determining errors incurred in making simplifications at a single scale, there are few techniques for integrated models which span several scales with complicated signalling. It is still unclear in such complex systems how errors and noise grow and are propagated, an important future issue.

7. Speculations Towards a Mathematical Theory

We have focused on modelling aspects at the various scales that characterise the system: molecular, cellular and tissue, as well as on the links between models at a specific scale with those at the lower and higher scales.

Modelling at each scale is an essential passage to multiscale approaches. The overall system is viewed as a network of several interacting subsystems each described by different models at different scales. Indeed, it is well understood that a multiscale description is essential to capture the complexity of biological systems in general.

This final section is devoted to the ambitious aim of outlining the conceptual paths towards the development of a mathematical theory related to the complex system under consideration. The various concepts proposed in what follows are essentially based on two key papers taken from the biological sciences which aim

not only to model very specific aspects, but also to put complex biological systems into a general mathematical framework.

The relevant objective of the mathematical research is analogous to that of the past two centuries when mathematics produced the formalisation of several phenomena in the science of physics. In other words, we aim to contribute to a preliminary reasoning on the genesis of a biological mathematical theory using paths followed, in the past, in the genesis of physical mathematical theories.

In detail we refer to the already cited papers by Hartwell *et al.*[103] and Hanahan and Weinberg.[102] The first paper[103] proposes the idea of a modular approach, where the whole system is decomposed into subsystems (modules), such that the identification of each module is related to the expression of specific biological functions. The modular approach has been developed in various papers[28,29,104]; our paper aims to indicate how the approach can be developed at a practical level.

The second paper[102] is focused on the analysis of the effect of genetic evolutionary mutations on the onset and progressive development of cancer towards stages with increasing malignancy.

The following subsections are devoted to identifying how the above two papers can contribute to the development of a mathematical biological theory and, in addition, how the analysis of mathematical problems, related to the application of models to the study of real biological phenomena, can take them into account.

7.1. *Perspective ideas for a mathematical biological theory*

Hartwell's theory indicates that we first need to identify the relevant biological functions that are expressed during the evolution of the system. Consequently, mathematical methods can provide the decomposition of the overall system into modules corresponding to the above functions.

According to the analysis of the preceding sections, it is understood that:

(i) The notion of function or purpose differentiates living systems in biology from those of inert matter. Biological functions have the ability to modify the conservation laws of classical mechanics and, in addition, can generate destructive and/or proliferating events;
(ii) The modular approach is applied to decompose complex biological systems into several modules that may, at least in some cases, be constituted by several elements which cooperatively express the above-mentioned biological functions;
(iii) Each module is related to a well-defined scale, namely to models that are characterised by a different mathematical structure;
(iv) Systems in biology cannot be simply observed and interpreted at a macroscopic level. A system constituted by millions of cells shows at the macroscopic level only the output of cooperative and organised behaviours which may not, or are not, singularly observed.

The paper by Hanahan and Weinberg,[102] in the context of item (i), reports how the relevant biological functions are related to genetic mutations, where undesired

corruptions are often transferred into the expression of function. Section 4 critically analysed various mathematical approaches to model the dynamics at the molecular scale with the attempt to understand how it affects dynamics and evolution at the higher scales.

As we have seen, biological events at the higher scale of cells and tissues depend on the dynamics at the molecular scale. Transferring the information from genes to cells is key to the derivation of a mathematical biological theory. This reasoning can be made precise by referring to the cellular scale.

The mathematical structure reported in Sec. 3 can generate specific models if the following parameters that, in general, depend on the microscopic states, are properly identified:

the encounter rate η_{hk};
the transition probability density $\mathcal{B}_{hk}$;
the population transition terms μ^i_{hk};
the proliferating/destructive terms μ_{hk}.

The physics of classical particles, whose dynamics is ruled by particle interaction models described by attractive–repulsive potentials, gives a useful analogy. Newtonian mechanics provides the necessary mathematical background to describe particle interactions by attraction–repulsion potentials of the interacting particles, or by mechanical collisions which preserve mass, momentum and energy. A theoretical description of the interaction potentials which govern pair interactions between particles completes the theory. In the case of the system under consideration, biology should contribute, by experiments and theoretical interpretations, to determine the form the outcome of cellular interactions as described by the above-mentioned interaction terms.

The mathematical models known in the literature have been obtained by assessing the above-mentioned terms by a simple phenomenological interpretation of physical reality and, in particular, of cellular interactions. A variety of models to describe immune competition with cancer cells has been reported in the book.[35] These models have shown the ability to describe several interesting phenomena all related to the parameters of the model. These parameters have a well-defined biological meaning.

As shown in Sec. 4, the structure of the equations used to describe tissue depends on the predominance of one of the three aspects of the biological dynamics, i.e. encounter rate between cells, mutations and proliferating/destructive events, with respect to the other two. Moreover, the structure of the mathematical equations modelling tissues may evolve in time due to the dynamics of genetic mutations.

Moreover, more than one scale is necessary to represent the system. A typical example is the modelling of angiogenesis.[80,81,132] Various modelling approaches can be found in the literature (see for example Refs. 174, 165, 166, 200 and 155). These papers offer interesting descriptions of particular biological phenomena and indicate that rarely is one scale only sufficient to model even particular components

of the system under consideration. A mathematical theory should identify for each subsystem, regarded as a module, the correct mathematical structure suitable to generate models. Such a structure may evolve in time, which is one of the main difficulties inherent in generating a mathematical biological theory.

7.2. *Mathematical problems*

The application of models to the analysis of real biological phenomena generates novel and challenging mathematical problems related to various issues, such as qualitative and computational analysis of the solutions, development of asymptotic methods to reach the higher scales from the lower ones, and so on.

The variety of mathematical problems is broad and covers several issues related to qualitative and computational analysis of the solutions to problems. Let us consider, to avoid over-generalisation, papers limited to the following classification:

(i) Free and moving boundary problems (that may also be related to therapeutical applications). See, for example, Cui and Friedman,[59] Friedman and Lolas,[85] Friedman,[86] Bertuzzi, Fasano and Gandolfi,[36,37] Tan and Guo,[182] Tao *et al.*[183,184] A general formulation of the problem can be found in the paper by De Angelis and Preziosi.[63]
(ii) Qualitative analysis of initial value problems related to models at the cellular scale. See, for example, De Angelis and Jabin,[61,62] Derbel,[64] Bellouquid and Delitala,[33,35] Micheler, Perthame and Ryzhik,[144] and Michel.[143]

The above research fields do not cover the whole variety of mathematical problems that have engaged applied mathematicians in this area. Indeed, a comprehensive review of all the problems in this area is beyond the scope of this paper. We have chosen to focus on a few key topics in the hope that they are sufficient to arouse the reader's interest. To summarise briefly:

— The biological system under consideration modifies its structure in time due to genetic mutations. Therefore, the mathematical structure of the model may change and should, at least in principle, be coupled with an evolution equation for genetic mutations.
— The system is made up of several interacting modules. Each module needs to be fully understood and suitably reduced before being integrated into the whole. A key problem here is to understand how noise and error propagate through the integrated whole.

While we have stressed the need for inter-disciplinary collaboration, we should not lose sight of the need for intra-disciplinary collaboration. For example, at a very abstract level, what we are really trying to do is to understand the spatio-temporal population dynamics of individuals with dynamically evolving internal states. This is very similar to the problems addressed by mathematical epidemiologists. An

intriguing future direction may be to use insights from that area to inform modelling approaches in mathematical oncology.

Future research will definitely refine and improve the existing models, while the analysis of the inherent mathematical problems will hopefully lead to new mathematics, allowing us to tackle problems presently beyond our technical abilities.

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