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Chapter 3

Innovative Models to Assess Multiple Myeloma Biology and the Impact of Drugs

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Additional information is available at the end of the chapter

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1. Introduction

Tumor and its embedding microenvironment form a unique, dynamic system, largely orchestrated by cellular players, including fibroblasts and endothelial cells (EC), and surrounding extracellular matrix (ECM) with its distinctive physical, biochemical, and biomechanical properties. There is a general consensus that, beyond genetic mutations and epigenetic modifications, the dialogue that occurs between tumor and its microenvironment, through soluble factors and molecular interactions, may affect tumor cells survival, growth, proliferation, response to chemical/physical factors, and lies the basis for metastatization to distant, specific organs. This theory was proposed by Paget in the 1880s [1], who underlined the need, for investigating and targeting tumor, to focus not only on the cancer cell, "the seed", but also on the "soil" where tumor homes and in which it derives its nutrients, oxygen and signals [2, 3]. Accordingly, tight links between tumor and surrounding microenvironment could determine the overall sensitivity to anti-cancer drugs and therefore represent an attractive therapeutic target [4].

Tumor microenvironment plays a critical role also in development and progression of haematological malignancies [5,6]. In this regard, Multiple Myeloma (MM) represents a paradigmatic condition [5,6]. Indeed, MM plasma cells almost exclusively home and thrive inside Bone Marrow (BM) microenvironment, which confers anti-apoptotic and pro-survival signals and resistance to drugs. In turn, tumor cell interactions with BM cells and matrix re-

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sult in re-shaping of microenvironment, and architectural changes involve in particular the vascular compartment [7].

The establishment of tight links between MM plasma cells and their microenvironment underlines the need for appropriate models for studying MM biology and predicting the impact of drugs.

In the present paper, we briefly summarize the role of BM microenvironment and, particularly, of MM associated angiogenesis, in MM pathogenesis, progression and prognosis. We then provide an overview of the currently available MM models, including animal models and a new three-dimensional (3D), gel-based, *in vitro* model of human MM microenvironment. Finally, we discuss the potential of RCCS™ bioreactor-based, dynamic 3D model systems (cell and tissue culture) to investigate critical aspects of human MM pathobiology and possible clinical applications. Advantages and limitations of each model, relative to MM investigation and assessment of drug sensitivity, are also considered.

2. Role of BM microenvironment and angiogenesis in MM progression and prognosis

MM is a B-cell tumor, characterized by clonal proliferation of malignant plasma cells inside the BM, production of a monoclonal paraprotein, and associated clinical features, including lytic bone lesions, renal insufficiency, hypercalcemia and anemia. It accounts for approximately 1% of neoplastic diseases and 13% of hematologic cancers. Albeit significant advances have been recently achieved in the treatment of MM, the disease still remains incurable, prompting the development of new therapeutic strategies [8].

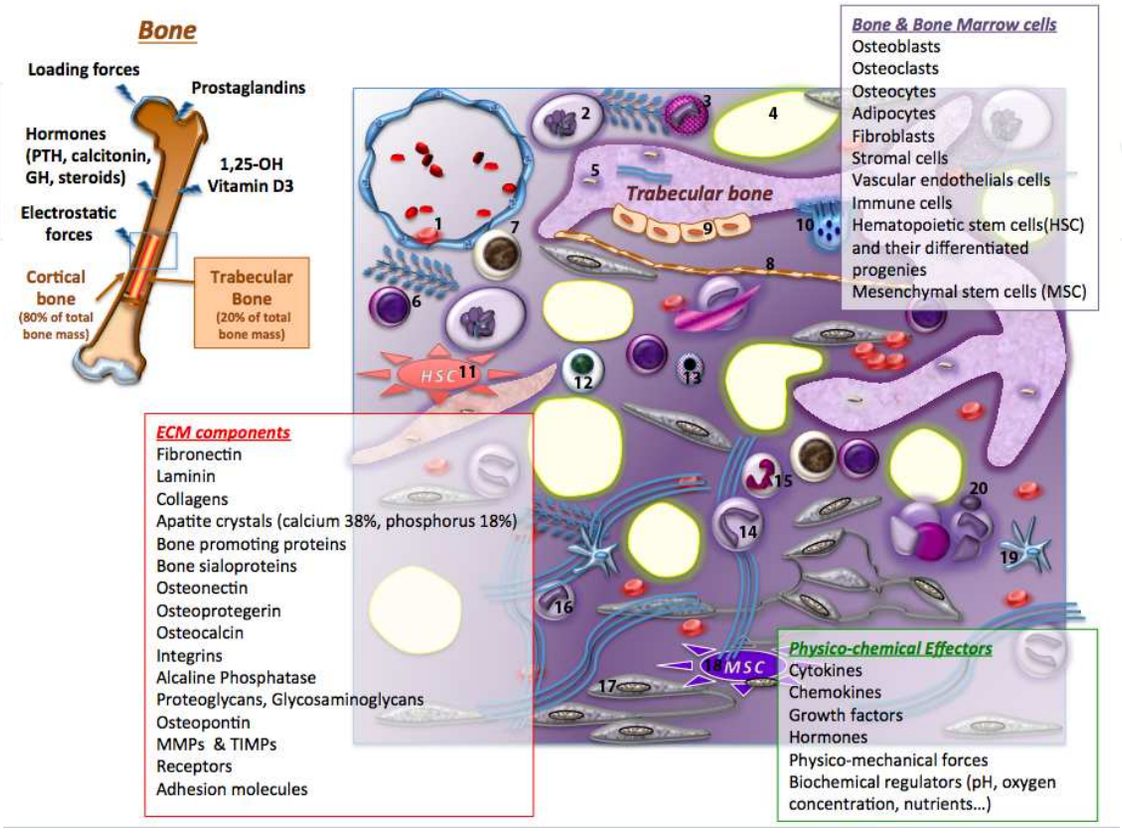
MM is thought to evolve from a pre-malignant syndrome known as Monoclonal Gammopathy of Uncertain Significance (MGUS), that progresses to smoldering (asymptomatic) myeloma and, finally, to symptomatic myeloma. In addition to genetic abnormalities accumulating in MM cells, BM microenvironment actively participates to the pathogenesis and progression of the disease. Indeed, host stromal components profoundly influence many steps of tumor progression, such as tumor proliferation, invasion, angiogenesis, metastasis, and even malignant transformation [9]. The BM, where MM cells specifically home, provides a highly specialized microenvironment, which optimally "soils" neoplastic plasma cells, and, in turn, is shaped by the interactions with MM cells [5,6,10].

BM microenvironment consists of a series of cellular components, including hematopoietic cells, immune cells, BM stromal cells (BMSC), osteoclasts, osteoblasts and endothelial cells (EC), all embedded in an extracellular matrix (ECM) (Fig.1).

MM cells specifically localize inside the BM milieu through the CXCR4/CXCL12-SDF1-alpha axis [11] and then interact with ECM and BM cellular components by means of adhesion

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molecules, including integrins. The complex interplay between MM cells and BM milieu, together with the ensuing pathogenetic events, are depicted in Fig. 2 (upper panel).



1: erithrocytes; 2: megacaryocytes; 3: basophils; 4: adipocytes; 5: osteocytes; 6: B lymphocytes; 7: monocytes; 8: lining osteoblasts; 9: osteoblasts; 10: osteoclasts; 11: hematopoietic stem cells "niche"; 12: T lymphocytes; 13: NK cells; 14: eosinophils; 15: neutrophils; 16: monocytes; 17: stromal cells; 18: mesenchymal stem cells "niche"; 19: dendritic cells; 20: thrombocytes (platelets).

Figure 1. Bone Marrow microenvironment. Bone homeostasis is the result of a complex network of stimuli, including hormones, vitamins and physico-mechanical forces. In addition to osteoblats and osteoclasts, which are responsible for bone deposition/resorption, BM microenvironment encompasses several cell types, like hematopoietic cells, endothelial cells and mesenchimal cells, all embedded in a complex extra-cellular-matrix (ECM).

Interactions between MM cells and ECM and cellular components (Fig. 2, lower panel) trigger the release of soluble factors, which, in turn, determine autocrine/paracrine loops of MM survival/proliferation and also promote osteoclastogenesis, defective immune functions and the "angiogenic switch", overall leading to MM cells growth, survival, and resistance to chemotherapeutic agents [10]. In particular, adhesion of MM cells to BMSC and to ECM components triggers anti-apoptotic signals and also the release of the pro-survival factor Interleukin (IL)-6. Moreover, MM plasma cells and BM stroma release osteoclast-acivating factors, including IL-1, IL-6, tumor necrosis factor (TNF)-α, RANK-L(Ligand) and Macrophage Inflammatory Protein (MIP)-1α. MM cells have also a unique ability to evade immune surveillance through several mechanisms, including impairment of cytotoxic activity and induction of dendritic cells dysfunction (Fig. 2).

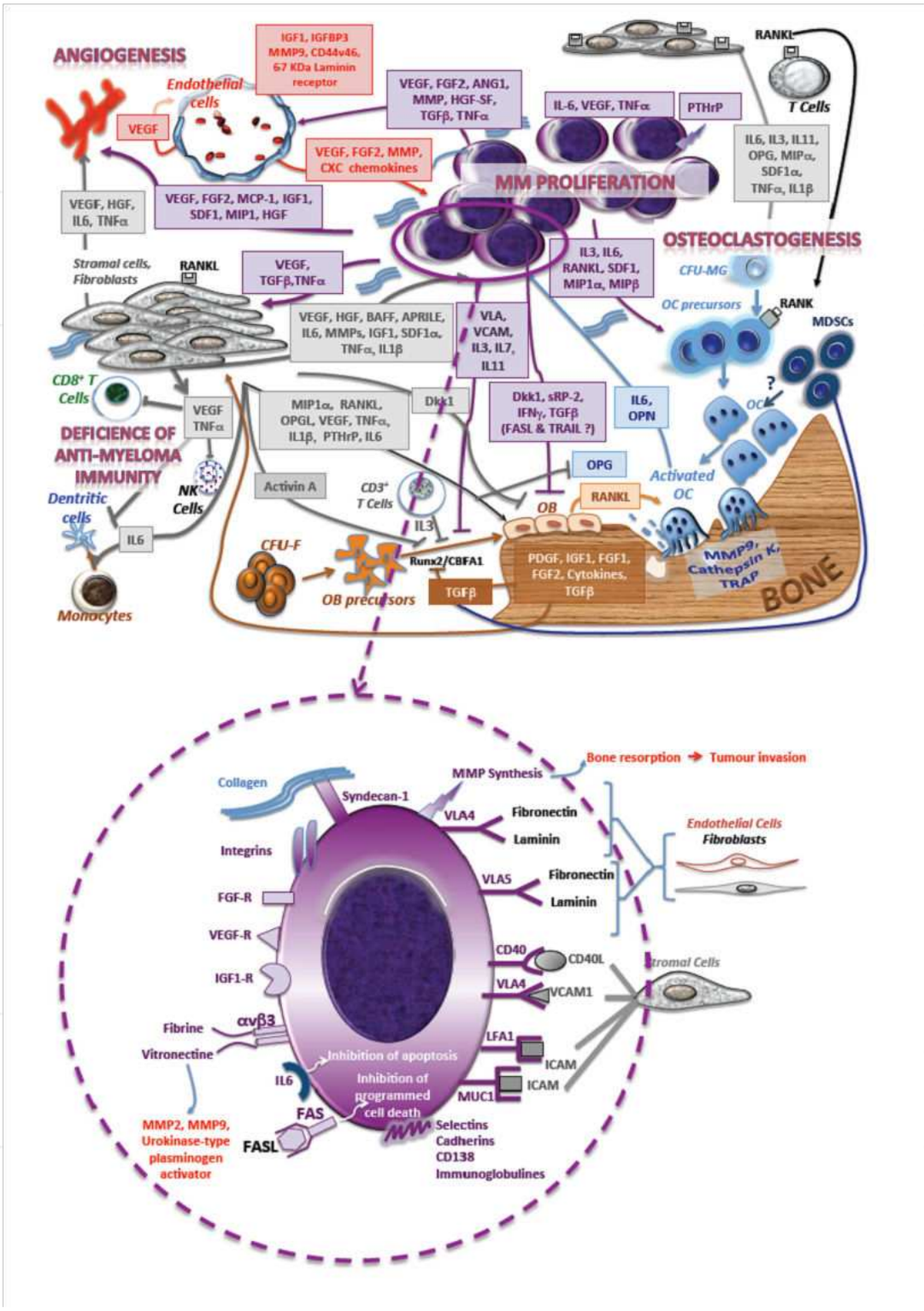


Figure 2. Interactions between MM cellsand BM microenviroment. Upper panel: schematic representation of MM cells inside BM microenvironment; the soluble factors involved in the major pathogenetic events, including tumor proliferation/survival, angiogenesis, osteoclastogenesis and defective immune function are depicted. Lower panel illustrates the major growth factor receptors and adhesion molecules used by MM plasma cells to interact with ECM and cellular components of BM microenvironment

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Angiogenesis, the sprouting of capillaries from existing blood-vessels, is a complex, dynamic and tightly regulated process, that occurs physiologically during normal growth, wound repair after injury and regeneration [12,13]. Angiogenesis is controlled by the balance between positive and negative regulators. In a tumor microenvironment, the exaggerate expression of pro-angiogenic cyto-chemokines starts the ''angiogenic switch'', leading to increased micro vessel density (MVD) [14]. The occurrence of an "angiogenic switch", responsible for the transition from the avascular "dormant" phase to the vascular phase of exponential tumor growth [15,16], has also been proposed for MM. Pro- and anti-angiogenic soluble molecules are produced and released by myeloma cells and components of microenvironment, including MMEC, stromal cells and inflammatory cells [17-19] (Fig.2 A, upper panel). Major angiogenic cytokines are VEGF-A, fibroblast growth factor (FGF) and hepatocyte growth factor (HGF). Both EC in general, and in particular MMEC, and MM cells secrete VEGF and express its receptors, thereby contributing to autocrine/paracrine pathways of tumor growth, survival and angiogenesis [19]. Finally, Angiopoietins (Angs, Ang-1 and-2) are important mediators in vasculature homeostasis and their circulating levels are considered of prognostic significance in MM [20].

Overall, BM angiogenesis in MM contributes to disease progression; accordingly, new anti-myeloma agents target not only MM cells, but also the microenvironment, and in particular vessels [21]. This notion is exemplified by the proteasome inhibitor Bortezomib (PS-341, Velcade), which has been approved for treatment of patients with relapsed and refractory MM and more recently used in front-line therapy for the disease. *In vitro*, proteasome inhibition by bortezomib causes apoptosis in both solid tumor and haematological malignancies, particularly MM [22]. More recently, Bortezomib has also been reported to affect viability of angiogenic EC, as shown in *in vitro* experimental conditions as well in animal models [23,24]. Notably, neither reliable biomarkers measurable *in vivo* nor *ex vivo* models of human BM microenvironment are currently available to assess the anti-angiogenic effect of drugs in MM patients.

3. Advantages of models which mimic tumor microenvironment exploiting the third dimension

Since BM microenvironment is of most importance in supporting myeloma cell growth and survival, experimental models of MM should provide insights into the mechanisms that, at molecular level, regulate the complex interplay between MM cells and biochemical and physical cues coming from BM ECM and cell components.

Traditional two-dimensional (2D) *in vitro* models (static culture of single cells kept as monolayer on flat, artificial surfaces) still represent the most popular models for *in vitro* studies, even if they present severe limitations, being unable to reproduce the behaviour and physiological responses of various normal and pathological cell types/tissues. It is now generally accepted that any attempt aimed at the generation of reliable and physiologically relevant *in vitro* tissue analogues, tumors included, should take into account the need of reproducing (or preserving) the specific characteristics of their original microenvironment, which in-

clude, in addition to tissue-specific multiple cellularity, biochemical and mechanical properties, also the three-dimensionality [25,26]. Since the pioneering studies of Bissell and colleagues [27], different groups, including ours, have demonstrated that significant differences exist between the biological behaviour and gene expression profiles of normal and transformed/tumor-derived cells maintained in culture with traditional (2D) culture methods, and that of cells kept in 3D culture (see, for example, 28-31), proving that 3D models can mimic *in vivo* conditions better than 2D systems [26,32,33].

Table 1 illustrates the principal characteristics of 3D *versus* conventional 2D *in vitro* models of differentiated and tumoral tissues, and their relevance to the *in vivo* situation.

Characteristics of the *in vitro* models	**2D conformation (on flat glass or plastic substrates)**	**3D conformation (cell spheroids, 3D artificial supports)**	**References**
In vitro models of differentiated tissues			
Architecture	Monolayer Lack of 3D physical cues	Multilayer Nano- and micro-topographies are recreated	34-37
Cell-milieu interaction	Unidirectional, passive fluid diffusion Lack of chemical gradients and reduced gas supply high ECM stiffness (more than 1 GPa)	Pluri-directional active fluid diffusion Gradients of nutrient and gas can be generated Efficient waste removal in dynamic bioreactors ECM stiffness lower than in 2D (variable from 1 to 100 kPa)	26, 38-41
Cell-cell interactions	Reduced interactions between neighbouring cells	Increased interactions between neighbouring cells	25
Cell morphology/ viability	Flat: geometrically-constrained baso-apical polarity Limited spatial distribution of adhesions to ECM Limited cell survival rate	Spheroid: free cell polarity guided by ECM Whole cell surface distribution of adhesions to ECM High cell survival rate	3142-44
Ability to mimic the physiological behaviour of cells in vivo	Lack the major physiological cues (biochemical, chemical, physical, mechanical) of the original tissue Low cell differentiation state and function	3D models are closer to the *in vivo* condition and number of *in vivo* cell/tissue features can be reproduced High differentiation state and functional competence ECM characteristics may vary, according to the culture model,	41, 45,46, 47

Characteristics of the *in vitro* models	2D conformation (on flat glass or plastic substrates)	3D conformation (cell spheroids, 3D artificial supports)	References
	Absent or abnormal neo-synthesized ECM (qualitatively and quantitatively)	from synthetic, natural and de-cellularized ECM, but 3D models are closer to the physiological context	
In vitro models of tumor tissues			
ECM-related cell motility and mechanobiology, compared to the in vivo situation	-	++	48 49
Cell organization	Organized	Disruption of tissue organization, as in i*n vivo* tumours	50
Gene expression	Higher growth-/ metabolic-related gene expression Activation of mitochondrial and ribosomal gene clusters Gene expression is, generally, quite different from *in vivo* tumours	Growth-arrest related genes are activated Closer to tumour tissue *in vivo*	51-53
Capability to reproduce specific morphological and behavioural characteristics of in vivo malignant cells	+/-	++	54,55
Responsiveness to surviving signal from ECM	+	++	55
Drug resistance (sensitivity)	Low (high)	High (low)	56-58
Capability to reproduce the complexity of tumour microenvironment	-	+/ +++	56 59,60

Table 1.

In an effort to reproduce *in vitro* the 3D specific microenvironment of the parental tissue, taking advantage of the rapid development of new technologies and tissue engineering techniques, an extremely wide variety of tissue models have been produced. The latter have already been successfully applied for investigating critical aspects of *in vivo* behaviour of a number of normal and tumoral cells (reviewed and discussed in 26). On this basis, 3D culture systems have been proposed as the most physiologically relevant *in vitro* models to investigate tumor development and behaviour [60-62]. Recently, this experimental approach has been also exploited for the study of MM-cell biology and sensitivity to therapeutic agents [63].

Within this context, 3D *in vitro* (cell-based)/*ex-vivo* (tissue-based) human-derived culture systems represent important tools to generate new approaches to the understanding of the molecular mechanisms of MM progression, essential prerequisites for the development of more effective interventional, diagnostic and prognostic strategies.

4. Murine models of MM

4.1. Subcutaneous xenograft models

The simplest way to generate an animal model of cancer consists in the injection of tumor cells into an immune-deficient mouse. This approach, known as the xenograft model, has been extensively employed for solid tumors [64,65] and then extended to MM. The xenograft model of MM consists in the subcutaneous injection of 1-2 x 10^7 human myeloma cells (from RPMI-8226, U266, ARH-77 or OPM-2 cell lines) into the flanks of Severe Combined Immune-Deficient (SCID), nonobese diabetic (NOD), SCID/NOD and SCID/beige, mice [66,67] (Fig.3A). The resulting plasmacytoma is palpable, and tumor burden measurable with a pair of caliper or, when lines are transduced with the eGFP-luc fusion gene, by bioluminescence imaging [68]. After harvesting, tumor mass is suitable for histological examination, allowing identification of vasculature and determination of cell proliferation/apoptosis. The model is currently used to assess the activity of new drugs on MM tumor growth and to establish the effective, minimally toxic, dose. As an example, this model has been employed to investigate the *in vivo* anti-myeloma effect induced by the mTOR inhibitor CCI-779 [69]. More recently, the efficacy of new inhibitors of the CXCR4/CXCL12 axis (AMD3100 and BKT140) [70], and of stressors of the endoplasmic reticulum (spicamycin analogue, KRN5500) [71], which inflict death of MM cells, have been demonstrated using the xenograft model. Besides mono-therapies, the model is suitable to evaluate the maximal effect, in terms of tumor volume reduction, obtainable with combined molecules [72].

While the xenograft model is extremely practical, particularly for drug testing, it still suffers from several limitations. In fact, it does not accurately mimic human disease, since myeloma cell lines do not behave as primary myeloma cells, more closely resembling the aggressive stage of plasma cell leukemia. More importantly, it fails to recapitulate the reciprocal interactions between MM cells and their microenvironment, which follow MM cell localization and retention inside the BM. As a result, drug efficacy can be over-estimated, lacking implanted MM cells the specific, proper human context of ECM and non-malignant accessory cells.

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Murine models of MM, including the 5TMM model, contribute to overcome this latter limitation.

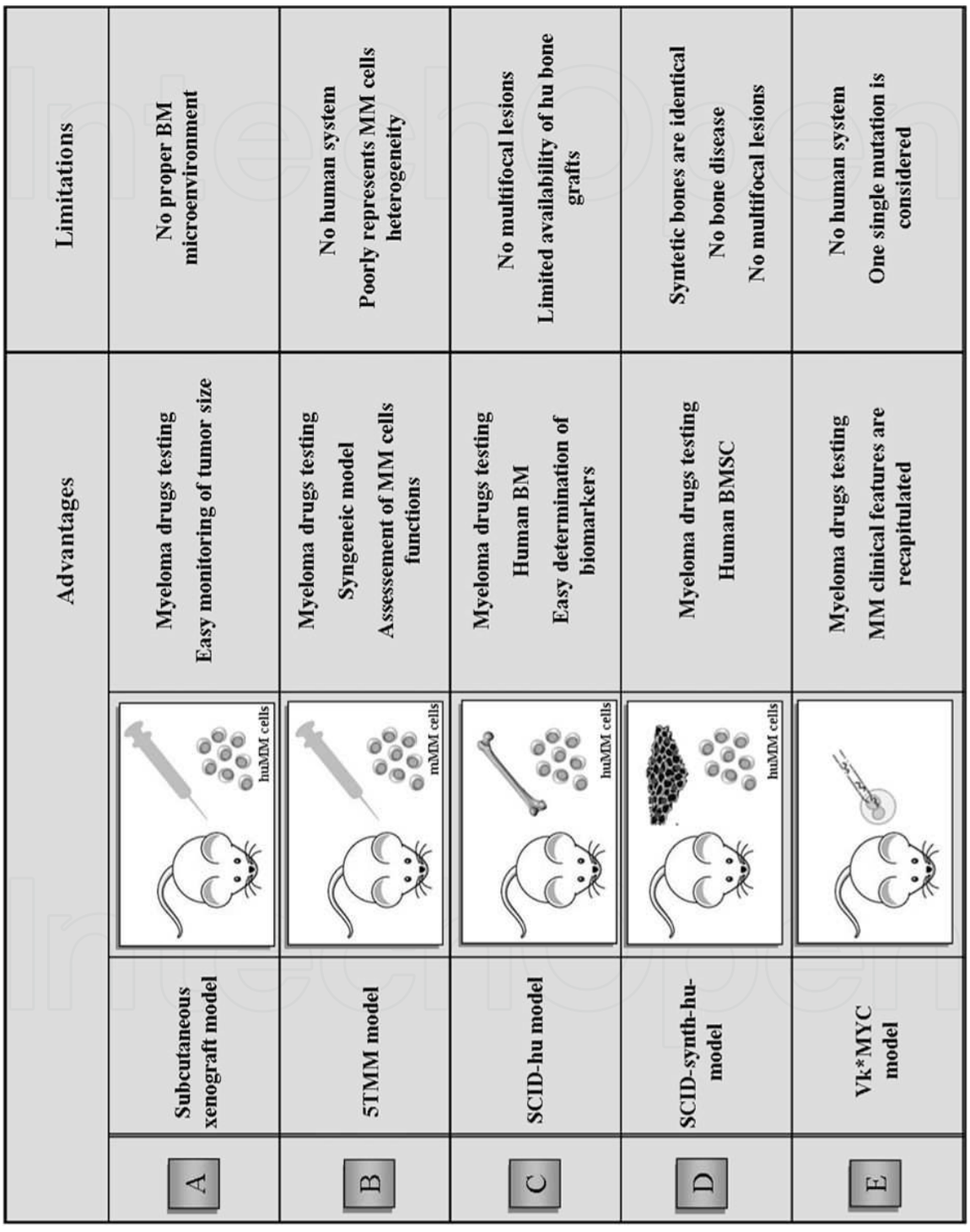


Figure 3. Schematic representation of currently available MM animal models. The major murine (m) and murine-human(hu) models together with their main advantages and limitations are depicted. Synth = synthetic polymeric scaffold; BMSC= Bone Marrow Stromal Cells; SCID=severe combined immune deficient.

4.2. 5TMM models

The 5T model has been developed in the late seventies upon injection of mice with syngeneic murine MM cells, spontaneously arising in elderly C57BL/KaLwRij mice [73,74]. The group of MM murine models collectively indicated as 5TMM mice comprises different types of mice, each bearing different tumor cells and having distinct characteristics (Figure 3B). The most commonly used, the 5T2MM and the 5T33MM models, display selective localization of cells in the BM, the presence of a serum M component and increased BM angiogenesis. The first one is characterized by moderate growth and development of osteolytic lesions more closely reproducing the human disease, while the second one displays a more aggressive behaviour with rapid growth [75].

Studies based on these models, substantially contributed by Karin Vanderkerken's group, have provided valuable insights into MM biology, and in particular on the mechanisms responsible for bone disease, MM-associated neoangiogenesis, and MM cell homing to the BM [75]. Indeed, taking advantage from these models, it has been possible to dissect the single steps which participate to the homing process, including chemo-attraction, adhesion, transendothelial migration and invasion, and also to identify the molecular pairs involved [75]. Moreover, these models allow the assessment of the impact of drugs on MM cells inside their proper microenvironment. In particular, the 5T2MM model allowed to unravel the anti-tumor activity, in addition to prevention of bone resorption, of the amino-biphosphonate zolendronic acid [76]. More recently, the novel 'second-generation' pyrimidyl-hydroxamic acid-based histone deacetylase inhibitor JNJ-26481585 was found to reduce tumor burden and also to affect angiogenesis and osteolysis [77].

A major limitation of the model is represented by the limited availability of different 5T cell lines, which fails to recapitulate the high variability both in terms of genetics and of tumor behaviour which characterize MM developing in humans. Moreover, the results obtained with 5T models should be interpreted with caution, given the potential differences in the biology of human vs murine myeloma.

4.3. SCID-hu and SCID-synth-hu models

In an attempt to "humanize" murine models, in 1997 Urashima established an *in vivo* model of human MM using SCID mice bilaterally implanted with human fetal bone grafts (SCID-hu mice) [78]. The purpose was to study the role of adhesion molecules which participate to human MM-BMSC interactions and regulate MM cell homing. The original experimental design consisted in the injection of MM cell lines (ARH-77, OCI-My5, U-266 or RPMI-8226) ($1x10^4$-10^5) into the BM cavity of the left bone implants in irradiated mice (Fig.3C). Human monoclonal MM cells grew within the human BM replacing the stroma and metastatized to the controlateral right bone implant, but not to murine bones or other murine organs [79], suggesting the existence of species-specific interactions. In myeloma-bearing mice, circulating human Ig were detectable and mice developed tubular nephropathy, due to light chains deposition, closely mirroring MM clinical manifestations and physiopathology [79]. The model was successfully employed to study the efficacy of thalidomide as an anti-myeloma drug, disclosing its anti-angiogenic properties [80]. The engraftment of IL-6-dependent

INA-6 cells in SCID-hu mice, but not in SCID mice, as well as their sensitivity to anti-myeloma agents, has also been documented [81].

More recently, Pierfrancesco Tassone and Filippo Causa developed the so-called "SCID-synth-hu" model (Figure 3D), based on the implantation of artificial bone scaffolds repopulated with human BMSC into SCID mice, followed by injection of purified MM cells from patients [82] (Fig. 3D). This model represents a further advancement over the previously described SCID-hu mouse (Fig. 3C). In fact, the use of 3D poly-β-caprolactone polymeric scaffolds, closely reproducing the micro-architecture of a human bone, overcomes the restricted availability of human fetal bones for implant, and also allows to perform studies in the context of an autologous setting [82].

As for SCID-hu models, in SCID-synth-hu mice injected human MM cells were found to optimally engraft the implanted "niche" and to interact with the human bone milieu, as demonstrated not only by histological and immunohistochemical analyses of the retrieved implants, but also by demonstration of immungloglobulin production *in vivo* [82].

Both systems thereby offer the possibility to investigate human MM cells-BM microenvironment interactions and to perform pre-clinical testing of anti-MM drugs in a clinically relevant context.

4.4. Transgenic models

MM cells accumulate a series of somatic mutations in the initiating and progressing phases of the disease [10], thus justifying development of genetically modified MM murine models, which recapitulate and explore the genetics of MM [83]. Recently, a model has been developed based on the enforced B cell lineage-directed transgene expression of XBP-1s [84]. XBP-1 is a major regulator of the Unfolded Protein Response (UPR) and plasma cell differentiation. Moreover, XBP-1 over-expression has been implicated in human carcinogenesis and tumor growth in solid tumors and also in MM [84]. XBP-1 transgenic mice spontaneously develop MGUS which progresses to MM, exhibiting remarkable clinical features common to human MM. In particular, BM involvement with clonal MM cells, serum M spike, bone lytic lesions and renal Ig deposition could be demonstrated [84].

Another model exploited the deregulated expression of Myc. Myc activation occurs in post-germinal center malignancies, including Burkitt's lymphoma, and is a common feature in MM; in particular its over-expression is generally considered of prognostic significance [85]. Mice engineered to express c-Myc under the control of mouse immunoglobulin kappa (IgK) light-chain gene–regulatory elements (Vk-Myc mice) were developed [86]. Myc is a strong oncogene, and its constitutive expression in early B cells of Vk-Myc mice led to a very aggressive lymphoma, with extra-medullary localization [86].

To create a transgenic mouse model more closely resembling human MM, in their elegant work Chesi and co-workers selected the C57Bl6 strain, genetically predisposed to develop MGUS, and generated a vector (Vk*Myc) containing a stop codon insertion in the human c-myc oncogene, which prevented its expression [87] (Fig. 3E). Myc could be then sporadically activated in post-germinal B cells as a result of somatic hypermutation, leading to the transi-

tion from the spontaneous monoclonal gammopathy to a disease that fully recapitulate the biological and clinical features of human MM. In fact, Vk*Myc mice are characterized by the accumulation of slowly proliferating plasma cells exclusively inside the BM. Moreover, high levels of monoclonal antibody are detectable and end-organ damage develops, including anemia, kidney failure and lytic bone disease [87]. The model was found to be highly predictive of the activity of anti-myeloma drugs [88], including those that target microenvironment, and may potentially help to select new agents for evaluation in clinical trials.

5. Human-derived models of MM

5.1. 3D *in vitro* /*ex-vivo* human-derived models of MM

Due to inter-species differences, animal models have incomplete predictive value for human MM disease and drug response. New models are, therefore, needed that more closely resemble the *in vivo* situation in patients. Reliable, human-derived *in vitro* models, able to reproduce myelomagenesis within the specificity of BM microenvironment, are therefore of extreme value.

Kirshner and her group have reconstructed, *in vitro,* human BM microenvironment, through the proper overlay of matrix components, on which isolated cells from BM aspirate of MM patients were seeded [63]. Cells spontaneously redistributed throughout the gel-matrix 3D substrate, mimicking human BM architecture and BM-MM interactions, thus providing a powerful tool for understanding the biology of MM [89]. Strikingly, reconstructed BM allowed the expansion of primary myeloma cells, including the putative stem cell fraction. Moreover, the model allowed the assessment of the impact of anti-MM drugs on distinct cellular compartments inside a 3D architecture [63].

5.2. 3D culture of human MM isolated cells and tissue explants in the microgravity-based RCCS™ bioreactor

It is well known that the metabolic requirements of complex 3D cell constructs are substantially higher than those needed for the maintenance of traditional cell monolayers (2D culture) kept in liquid media under static conditions. Dynamic bioreactors were primarily developed to modulate mass transfer, a crucial element for guaranteeing gas/nutrient supply and waste elimination, essential factors for maintaining cell viability within large 3D cell/tissue masses. Despite a wide array of fluid-dynamic bioreactors has been devised [47,90], the low-shear environment and optimal mass transfer, needed for the long-term culture of functional 3D tissue constructs and explants, were attained only with the introduction of the microgravity-based *Rotary Cell Culture System* (RCCS™, Synthecon Inc., USA) bioreactors (91,92; a vast literature is also available at http://www.synthecon.com). The relevance of this technology in enabling the long-term culture of complex tissue-like engineered 3D bio-constructs and tissue explants of various origin has been demonstrated also by our group, and, namely, in the case of bone [31,47.93].

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On this basis, we successfully employed the microgravity-based RCCS™ technology for the generation (and long-term maintenance) of viable human-derived MM tissue explants and 3D cell constructs. Fig. 4 shows the culture chamber of the RCCS™ microgravity-based bioreactor, and histo-morphological images of the *ex-vivo* models of human MM developed by our group. Isolated cells from the RPMI myeloma cell line, kept in Bioreactor, spontaneously self–aggregated forming spheroid-like structures which retained viability and were identifiable with the specific anti-CD38 monoclonal antibody (Fig.4B).

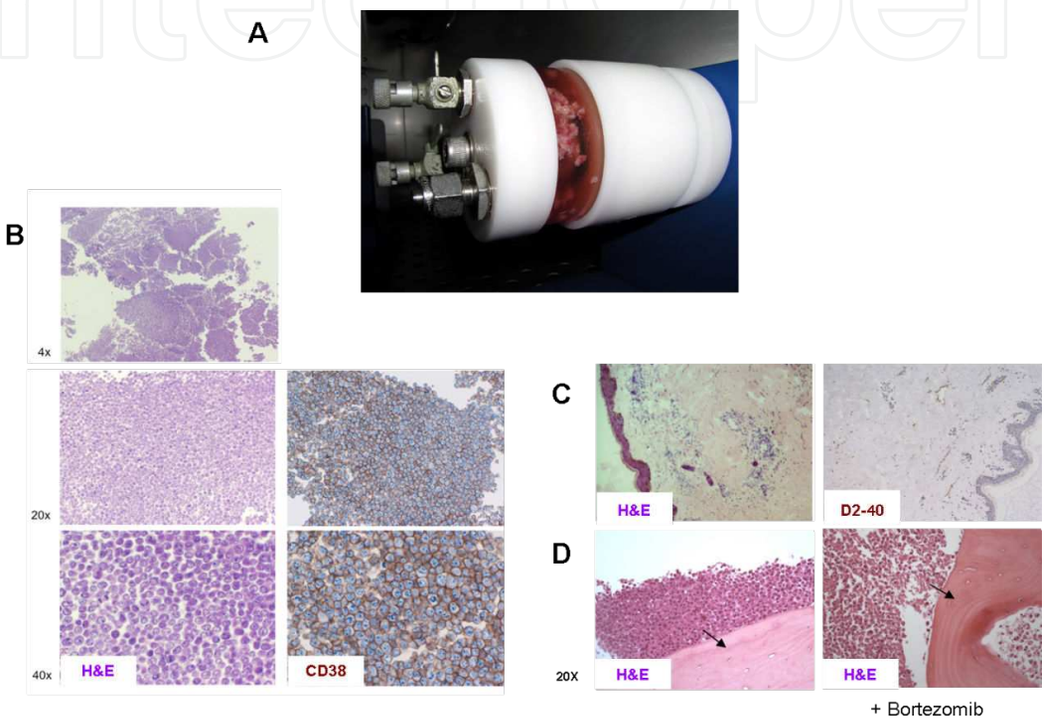


Figure 4. RCCS ™-based 3D *ex-vivo* models of MM developed by our group. A: Detail of the culture chamber of the RCCS™ microgravity-based bioreactor; **B**: Monotypic 3D multi-cellular spheroids (RPMI cell line) cultured for 1 week in the RCCS™ bioreactor (H&E staining, left panels; CD38 staining, right panels); **C:** 3D tissue culture of skin biopsies (1 week) showing intact architecture and identifiable blood and lymphatic (D2-40+) vessels **D**: MM tissue explants cultured for 3 days in the RCCS™ bioreactor (H&E staining), in the absence or presence of Bortezomib, the latter showing plasma cells death. Arrows indicate bone lamellae.

The suitability of our method for the culture of human tissue samples was, firstly, proved by using skin biopsies, which retained intact epidermal and dermal architecture, including keratin stratum and skin annexes. Moreover, both blood and lymphatic vasculature was identifiable and exhibited normal morphology, in particular patent lumen and complete endothelial lining (Fig.4C). The 3D culture of thick sections of human MM tissue explants fully preserved tissue architecture and microenvironment integrity (Fig.4D) for extended periods of time. Moreover, the system was suitable for the assessment of drug sensitivity, not only of tumor compartment, but also of angiogenic vessels (Fig.4D). Indeed, quantification of MVD in treated specimens could represent a unique method to assess the anti-angiogenic effect of a drug in human samples *ex vivo*. Finally, specialized functions of both MM cells and their microenvironment, including beta-2 microglobulin and cytokine release and met-

alloproteases activities, could be also assessed (M. Ferrarini *et al*., *submitted*). Overall, these observations suggest that the 3D culture model in Bioreactor can be exploited as a novel translational tool, allowing prospective pre-clinical toxicity and drug efficacy testing in individual patients.

6. Conclusions

A major challenge in cancer biology and cancer therapy relies in the availability of suitable models that recapitulate the complex tumor-host interplay and responsiveness to drugs. This is especially true for MM, where the existence of tight links between MM cells and BM microenvironment has hampered for long the development of adequate animals and *in vitro* models. Recently, innovative murine and chimeric *in vivo* models have been developed, which allowed both to investigate MM physiopathology and to perform drugs testing. On the other hand, the exploitation of novel technologies for *ex-vivo* 3D culturing of human MM samples is emerging as a tool to properly investigate its pathogenetic mechanisms (and interactions) within a human context, and also to predict response to drugs in individual patients.

The availability of more and more sophisticated systems is expected to pave the way to a deeper understanding of pathogenetic events and also to development of novel patients-tailored therapeutic strategies.

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References

[1] Paget S. The distribution of secondary growths in cancer of the breast. The Lancet 1889;1: 571–573.

[2] Witz IP. The tumor microenvironment: the making of a paradigm. Cancer Microenvironment 2009;2(Suppl. 1):9-17.

[3] Weber CE, Kuo PC. The tumor microenvironment. Journal of Surgical Oncology 2012;21(3): 172-177.

[4] Correia AL, Bissell MJ. The tumor microenvironment is a dominant force in multidrug resistance. Drug Resistance Update 2012;15(1-2):39-49.

[5] Tripodo C, Sangaletti S, Piccaluga PP, Prakash S, Franco G, Borrello I, Orazi A, Colombo MP, Pileri SA.The bone marrow stroma in hematological neoplasms: a guilty bystander. Nature Reviews Clinical Oncology 2011;8(8):456-466.

[6] Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies Blood 2009;114:3367-375.

[7] Vacca A, Ribatti D. Bone Marrow angiogenesis in Multiple Myeloma. Leukemia 2006;20:193-199.

[8] Palumbo A; Anderson K. Multiple Myeloma. New. English Journal of Medicine 2011;364:1046-1060.

[9] Dvorak HF, Weaver VM, Tlsty TD, Bergers G. Tumor microenvironment and progression. Journal of Surgical Oncology 2011;103:468-474.

[10] Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nature Reviews Cancer 2007;7:585-598.

[11] Alsayed Y, Ngo H, Runnels J, Leleu X, Singha UK, Pitsillides CM, Spencer JA, Kimlinger T, Ghobrial JM, Jia X, Lu G, Timm M, Kumar A, Côté D, Veilleux I, Hedin KE, Roodman GD, Witzig TE, Kung AL, Hideshima T, Anderson KC, Lin CP, Ghobrial IM. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. Blood. 2007;109(7):2708-17.

[12] Semenza GL. Angiogenesis in ischemic and neoplastic disorders. Annual Review of Medicine2003;54:17-28

[13] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249-257.

[14] Moserle L, Amadori A, Indraccolo S. The angiogenic switch: implications in the regulation of tumor dormancy. Current Molecular Medicine 2009;9:935-941.

[15] Ramanujan S, Koenig GC, Padera TP, Stoll BR, Jain RK. Local imbalance of proangiogenic and antiangiogenic factors: a potential mechanism of focal necrosis and dormancy in tumors. Cancer Research 2000; 60:1442–1448.

[16] Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nature Reviews Cancer 2003;3:401-410.

[17] Di Raimondo F. Angiogenesis in hematology: a field of active research. Leukemia Research 2003;27:571-573.

[18] Vacca A, Ribatti D. Bone marrow angiogenesis in multiple myeloma. Leukemia 2006;20:193-199.

[19] Pour L, Svachova H, Adam Z, Almasi M, Buresova L, Buchler T, Kovarova L, Nemec P, Penka M, Vorlicek J, Hajek R. Levels of angiogenic factors in patients with multiple myeloma correlate with treatment response. Annals of Hematology 2010; 89:385-389.

[20] Terpos E, Anargyrou K, Katodritou E, Kastritis E, Papatheodorou A, Christoulas D, Pouli A, Michalis E, Delimpasi S, Gkotzamanidou M, Nikitas N, Koumoustiotis V, Margaritis D, Tsionos K, Stefanoudaki E, Meletis J, Zervas K, Dimopoulos MA, Greek Myeloma Study Group, Greece. Circulating angiopoietin-1 to angiopoietin-2 ratio is an independent prognostic factor for survival in newly diagnosed patients with multiple myeloma who received therapy with novel antimyeloma agents. International Journal of Cancer 2012;130(3):735-742.

[21] Ferrarini M, Ferrero E. Proteasome inhibitors and modulators of angiogenesis in multiple myeloma. Current Medicinal Chemistry 2011;18(34):5185-5195.

[22] Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Research 2001;61(7):3071-3076.

[23] Roccaro AM, Hideshima T, Raje N, Kumar S, Ishitsuka K, Yasui H, Shiraishi N, Ribatti D, Nico B, Vacca A, Dammacco F, Richardson PG, Anderson KC. Bortezomib mediates antiangiogenesis in multiple myeloma via direct and indirect effects on endothelial cells. Cancer Research 2006;66(1):184-191.

[24] Belloni D, Veschini L, Foglieni C, Dell'Antonio G, Caligaris-Cappio F, Ferrarini M, Ferrero E. Bortezomib induces autophagic death in proliferating human endothelial cells. Experimental Cell Research 2010;316(6):1010-1018.

[25] Pampaloni, F., Reynaud, EG. & Stelzer, EH. (2007). The third dimension bridges the gap between cell culture and live tissue. Nature Reviews. Molecular Cell Biology, Vol. 8, No. 10, (October 2007), pp. 839-845, ISSN 1471-0080

[26] Mazzoleni, G., Di Lorenzo, D. & Steimberg, N. (2009) Modelling tissues in 3D: the next future of pharmaco-toxicology and food research? Genes and Nutrition, Vol. 4, No. 1 (March 2009), pp. 13-22, ISSN 1555-8932

[27] Roskelley CD, Desprez PY, Bissell MJ (1994) Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. Proc Natl Acad Sci USA 91:12378–12382.

[28] Lee GY, Kenny PA, Lee EH, Bissel MJ, (2007) Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods 4:359-365.

[29] Chang, TT. & Hughes-Fulford, M. (2009). Monolayer and Spheroid Culture of Human Liver Hepatocellular Carcinoma Cell Line Cells Demonstrate Distinct Global Gene Expression Patterns and Functional Phenotypes. Tissue Engineering: Part A, Vol. 15, No. 3, pp. 559-567, ISSN 1557-8690

[30] Pickl M & CH Ries (2009) Comparison of 3D and 2D tumor models reveals enhanced HER2 activation in 3D associated with an increased response to trastuzumab. Oncogene 28:461-468.

[31] Steimberg, N., Boniotti, J. & Mazzoleni, G. (2010). 3D culture of primary chondrocytes, cartilage, and Bone/cartilage explants in simulated microgravity. In: Methods in Bioengineering: Alternative Technologies to Animal Testing, Maguire and Novak, pp. 205- 212, ISBN 978-1-60807-011-4, Boston, USA

[32] Birgersdotter A, Sandberg R, Ernberg I (2005) Gene expression perturbation in vitro - a growing case for three-dimensional (3D) culture systems. Semin Cancer Biol 15:405–412.

[33] Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, Polverini PJ, Mooney DJ (2007) Engineering tumors with 3D scaffolds. Nat Methods 4:855–860.

[34] Yim EK, Darling EM, Kulangara K, Guilak F, Leong KW. Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. Biomaterials. 2010;31(6):1299-1306.

[35] Bierwolf J, Lutgehetmann M, Feng K, Erbes J, Deichmann S, Toronyi E, Stieglitz C, Nashan B, Ma PX, Pollok JM. Primary rat hepatocyte culture on 3D nanofibrous polymer scaffolds for toxicology and pharmaceutical research. Biotechnology and bioengineering 2011;108(1):141-150.

[36] Yoshii Y, Waki A, Yoshida K, Kakezuka A, Kobayashi M, Namiki H, Kuroda Y, Kiyono Y, Yoshii H, Furukawa T, Asai T, Okazawa H, Gelovani JG, Fujibayashi Y. The use of nanoimprinted scaffolds as 3D culture models to facilitate spontaneous tumor cell migration and well-regulated spheroid formation. Biomaterials. 2011; 32(26): 6052-6058.

[37] Kuo SM, Chiang MY, Lan CW, Niu GC-C, Chang SJ. Evaluation of nanoarchitectured collagen type II molecules on cartilage engineering. 2012; Journal of biomedical materials research. Part A. 2012:00A:000–000.

[38] Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophysical journal. 2000; 79(1):144-152.

[39] Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, Weaver VM. (2005) Tensional homeostasis and the malignant phenotype. Cancer Cell 8: 241–254.

[40] Schindler M, Nur-E-Kamal A, Ahmed I, Kamal J, Liu HY, Amor N, Ponery AS, Crockett DP, Grafe TH, Chung HY, Weik T, Jones E, Meiners S. Living in three dimensions: 3D nanostructured environments for cell culture and regenerative medicine. Cell Biochemistry and Biophysics. 2006;45(2):215-227.

[41] Mazzoleni, G., Boukhechba, F., Steimberg, N., Boniotti, J., Bouler, JM. & Rochet, N. (2011). Impact of the dynamic culture condition in the RCCS™ bioreactor on a three-dimensional model of bone formation. Procedia Engineering, Vol. 10, pp. 3662-3667, ISSN 1877-7058

[42] Gruber HE, Hanley EN Jr. Human disc cells in monolayer vs 3D culture: cell shape, division and matrix formation. BMC musculoskeletal disorders. 2000;1:1.

[43] Lin RZ, and Chang HY. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. Biotechnol J. 2008;3(9-10):1172-1184.

[44] Ortinau S, Schmich J, Block S, Liedmann A, Jonas L, Weiss DG, Helm CA, Rolfs A, Frech MJ. Effect of 3D-scaffold formation on differentiation and survival in human neural progenitor cells. Biomed Eng Online. 2010 Nov 11;9:70.

[45] Campbell JJ, and Watson CJ. Three-dimensional culture models of mammary gland. Organogenesis. 2009; 5(2): 43-49.

[46] Ross AM, Jiang Z, Bastmeyer M, Lahann J. Physical aspects of cell culture substrates: topography, roughness, and elasticity. Small. 2012;8(3):336-355.

[47] Mazzoleni, G. & Steimberg, N. (2012) New models for the in vitro study of liver toxicity: 3D culture systems and the role of bioreactors. In"The continuum of Health Risk Assessment", edited by Dr. M.G. Tyshenko; Intech Open Access Publisher, Rijeka, Croatia; chapter 8, pp. 161-194; ISBN 978-953-51-0212-0.

[48] Ulrich TA, de Juan Pardo EM, Kumar S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. Cancer Research; 2009; 69: 4167–4174.

[49] Pathak A. and Kumar S. Independent regulation of tumor cell migration by matrix stiffness and confinement. Proceedings of the National Academy of Sciences (PNAS) (2012); 109 (26): 10334-10339

[50] Bissell MJ, Radisky DC, Rizki A, Weaver VM, Petersen OW. The organizing principle: microenvironmental influences in the normal and malignant breast. Differentiation. 2002;70(9-10):537-546.

[51] Debnath J, and Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. Nature Review Cancer. 2005;5(9):675-688

[52] Sandberg R, and Ernberg I. The molecular portrait of in vitro growth by meta-analysis of gene-expression profiles. Genome Biol. 2005; 6(8):R65

[53] Härmä V, Virtanen J, Mäkelä R, Happonen A, Mpindi J-P, Knuuttila M, Kohonen P, Lötjönen j, Kallioniemi O, Nees M. A Comprehensive Panel of Three-Dimensional Models for Studies of Prostate Cancer Growth, Invasion and Drug Responses. 2010, PLoS ONE 5(5): e10431

[54] Petersen OW, R.nnov-Jessen L, Howlett AR, Bissell MJ: Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proceedings of the National Academy of Sciences USA 1992, 89:9064-9068.

[55] Smalley KS, Lioni M, Herlyn M. Life isn't flat: taking cancer biology to the next dimension In vitro Cellular & Developmental Biology 2006a; 42(8-9): 242-247.

[56] Fischbach C, Kong HJ, Hsiong SX, Evangelista MB, Yuen W, Mooney DJ. Cancer cell angiogenic capability is regulated by 3D culture and integrin engagement. Proc Natl Acad Sci U S A. 2009 Jan 13;106(2):399-404.

[57] Horning JL, Sahoo SK, Vijayaraghavalu S, Dimitrijevic S, Vasir JK, Jain TK, Panda AK, Labhasetwar V. 3-D tumor model for in vitro evaluation of anticancer drugs. Mol Pharm. 2008;5:849-862

[58] Chitcholtan K, Sykes PH and Evans JJ. The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. Journal of Translational Medicine 2012, 10:38

[59] Sasser AK, Mundy BL, Smith KM, Studebaker AW, Axel AE, Haidet AM, Fernandez SA, Hall BM. Human bone marrow stromal cells enhance breast cancer cell growth rates in a cell line-dependent manner when evaluated in 3D tumor environments. Cancer Lett. 2007 Sep 8;254(2):255-264.

[60] Yamada KM & Cukierman E (2007) Modeling tissue morphogenesis and cancer in 3D. Cell, 130:601-610.

[61] Kim JB. Three-dimensional tissue culture models in cancer biology. Seminars in Cancer Biology (2005)15: 365–377

[62] Hutmacher DW, Loessner D, Rizzi S, Kaplan DL, Mooney DJ, Clements JA (2010). Can tissue engineering concepts advance tumor biology research? Trends in Biotechnology, Vol.28 No.3, pp. 125-133

[63] Kirshner J, Thulien KJ, Martin LD, Debes Marun C, Reiman T, Belch AR, Pilarski LM. (2008) A unique three-dimensional model for evaluating the impact of therapy on multiple myeloma. Blood Vol. 112(7):2935-2945.

[64] Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Research 2006;66(7):3351-3354.

[65] Teicher BA. Tumor models for efficacy determination. Molecular Cancer Therapeutics 2006;5(10):2435-2443.

[66] Tong AW, Huang YW, Zhang BQ, Netto G, Vitetta ES, Stone MJ. Heterotransplantation of human multiple myeloma cell lines in severe combined immunodeficiency (SCID) mice. Anticancer Research 1993;13(3):593-597.

[67] Mitsiades CS, Anderson KC, Carrasco DR. Mouse models of human myeloma. Hematology/Oncology Clinics of North America 2007;21(6):1051-1069.

[68] Wu KD, Zhou L, Burtrum D, Ludwig DL, Moore MA. Antibody targeting of the insulin-like growth factor I receptor enhances the anti-tumor response of multiple myeloma to chemotherapy through inhibition of tumor proliferation and angiogenesis. Cancer Immunology, Immunotherapy 2007;56(3):343-57

[69] Frost P, Moatamed F, Hoang B, Shi Y, Gera J, Yan H, Frost P, Gibbons J, Lichtenstein A. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. Blood 2004;104(13):4181-4187.

[70] Beider K, Begin M, Abraham M, Wald H, Weiss ID, Wald O, Pikarsky E, Zeira E, Eizenberg O, Galun E, Hardan I, Engelhard D, Nagler A, Peled A. CXCR4 antagonist 4F-benzoyl-TN14003 inhibits leukemia and multiple myeloma tumor growth. Experimental Hematology 2011;39(3):282-292.

[71] Miki H, Ozaki S, Nakamura S, Oda A, Amou H, Ikegame A, Watanabe K, Hiasa M, Cui Q, Harada T, Fujii S, Nakano A, Kagawa K, Takeuchi K, Yata K, Sakai A, Abe M, Matsumoto T. KRN5500, a spicamycin derivative, exerts anti-myeloma effects through impairing both myeloma cells and osteoclasts. British Journal of Haematology 2011;155(3):328-39.

[72] Mirandola L, Yu Y, Chui K, Jenkins MR, Cabos E, John CM, Chiriva-Internati M. Galectin-3C inhibits tumor growth and increases the anticancer activity of bortezomib in a murine model of human multiple myeloma. PLoS One 2011;6(7):e21811.

[73] Radl J, Hollander CF, Van den Berg P, De Glopper E. Idiopathic paraproteinaemia I. Studies in an animal model--the ageing C57BL/KaLwRij mouse. Clinical & Experimental Immunology 1978;33(3):395-402.

[74] Radl J. Age-related monoclonal gammapathies: clinical lessons from the aging C57BL mouse. Immunology Today 1990;11(7):234-236.

[75] Menu E, Asosingh K, Van Riet I, Croucher P, Van Camp B, Vanderkerken K. Myeloma cells (5TMM) and their interactions with the marrow microenvironment. Blood Cells, Molecule and Disease 2004;33(2):111-119.

[76] Croucher PI, De Hendrik R, Perry MJ, Hijzen A, Shipman CM, Lippitt J, Green J, Van Marck E, Van Camp B, Vanderkerken K. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival, Journal of Bone and Mineral Research 2003;18(3):482–492.

[77] Deleu S, Lemaire M, Arts J, Menu E, Van Valckenborgh E, King P, Vande Broek I, De Raeve H, Van Camp B, Croucher P, Vanderkerken K. The effects of JNJ-26481585, a novel hydroxamate-based histone deacetylase inhibitor, on the development of multiple myeloma in the 5T2MM and 5T33MM murine models. Leukemia 2009;23(10): 1894-1903.

[78] Urashima M, Chen BP, Chen S, Pinkus GS, Bronson RT, Dedera DA, Hoshi Y, Teoh G, Ogata A, Treon SP, Chauhan D, Anderson KC. The development of a model for the homing of multiple myeloma cells to human bone marrow. Blood 1997;90(2): 754-765

[79] Yaccoby S, Barlogie B, Epstein J. Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations. Blood 1998;92(8):2908-2913.

[80] Yaccoby S, Johnson CL, Mahaffey SC, Wezeman MJ, Barlogie B, Epstein J. Antimyeloma efficacy of thalidomide in the SCID-hu model. Blood 2002;100(12):4162-4168.

[81] Tassone P, Neri P, Carrasco DR, Burger R, Goldmacher VS, Fram R, Munshi V, Shammas MA, Catley L, Jacob GS, Venuta S, Anderson KC, Munshi NC. A clinically relevant SCID-hu in vivo model of human multiple myeloma. Blood 2005;106(2): 713-716.

[82] Calimeri T, Battista E, Conforti F, Neri P, Di Martino MT, Rossi M, Foresta U, Piro E, Ferrara F, Amorosi A, Bahlis N, Anderson KC, Munshi N, Tagliaferri P, Causa F, Tassone P. A unique three-dimensional SCID-polymeric scaffold (SCID-synth-hu) model for in vivo expansion of human primary multiple myeloma cells. Leukemia 2011;25(4):707-711.

[83] DeWeerdt S. Animal models: Towards a myeloma mouse. Nature 2011;480(7377):S38-9

[84] Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS, Munshi N, Horner J, Ivanova EV, Protopopov A, Anderson KC, Tonon G, DePinho RA. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell 2007;11(4):349-360

[85] Zhan F, Tian E, Bumm K, Smith R, Barlogie B, Shaughnessy J Jr. Gene expression profiling of human plasma cell differentiation and classification of multiple myeloma based on similarities to distinct stages of late-stage B-cell development. Blood 2003;101(3):1128-1140

[86] Robbiani DF, Colon K, Affer M, Chesi M, Bergsagel PL. Maintained rules of development in a mouse B-cell tumor. Leukemia 2005;19(7):1278-1280

[87] Chesi M, Robbiani DF, Sebag M, Chng WJ, Affer M, Tiedemann R, Valdez R, Palmer SE, Haas SS, Stewart AK, Fonseca R, Kremer R, Cattoretti G, Bergsagel PL. AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. Cancer Cell 2008;13(2):167-180.

[88] Chesi M, Matthews GM, Garbitt VM, Palmer SE, Shortt J, Lefebure M, Stewart AK, Johnston RW, Bergsagel PL. Drug response in a genetically engineered mouse model of multiple myeloma is predictive of clinical efficacy. Blood. 2012;120(2):376-85.

[89] Jelinek DF. Myeloma research goes 3D. Blood 2008;112(7):2600-2601.

[90] Martin Y. & Vermette P. (2005) Bioreactors for tissue mass culture: design, characterization, and recent advances. Biomaterials; 26:7481-503.

[91] Mazzoleni, G. & Steimberg, N. (2010) 3D culture in microgravity: a realistic alternative to experimental animal use. Alternatives to Animal Experimentation (ALTEX), Vol. 27, special issue, pp. 321-324, ISSN 0946-7785

[92] Barzegaru A, & Saei AA (2012) An update to space biomedical research: tissue engineering in microgravity bioreactors. Bioimpacts, Vol.2(1), pp. 23-32

[93] Cosmi, F., Steimberg, N., Dreossi, D. & Mazzoleni, G. (2009). Structural analysis of rat bone explants kept in vitro in simulated microgravity conditions. Journal of the Mechanical Behavior of Biomedical Materials, Vol. 2, No. 2, (April 2009), pp. 164-172

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