
Manuela Cassotta1, Francesca Pistollato2 and Maurizio Battino1,3,4

1Nutrition and Food Science Group, Department of Analytical and Food Chemistry, CTPACA, CACTI, University of Vigo, Vigo, Spain; 2European Commission, Joint Research Centre (JRC), Ispra, Italy; 3International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang, China; 4Department of Clinical Sciences, Faculty of Medicine, Polytechnic University of Marche, Ancona, Italy

Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease characterized by progressive bone and cartilage destruction, functional impairment and long-term disability. Despite RA has been described in the medical literature for over two hundred years, the underlying etiology and pathophysiology are insufficiently understood. Current treatment of RA is mainly empirical or based on drugs that interfere with generic steps in immune response, with limited efficacy and/or significant side effects. Much of RA research has been traditionally based on animals and simplistic in vitro models, which have been shown to poorly recapitulate human RA etiopathogenesis and drug responses. A revolution in science and technology has produced a new generation of more relevant and predictive tools. These tools, which include patient-derived cells, innovative 3D cell culture systems, computational analyses and models, together with -omics and large-scale epidemiological studies represent novel and exciting equipment to enhance and forward RA research in a human biology-based perspective. After considering some pitfalls and flaws of traditional models, in this review we present a list of the novel available tools applicable to design a human-oriented RA research, while fostering the need for a more holistic and preventive approach to the disease. The goal of this review is to stimulate a discussion both at scientific and public level on the need to explore new avenues in RA research and to support a paradigm-shift from animal-based towards human biology-based systems, to better understand human pathophysiology, and to develop more effective targeted therapies for personalized treatment and prevention.

1 Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune disease of unknown etiology that affects the connective tissue. RA is characterized by chronic synovitis, diarthrodial joints inflammation and various degrees of bone and cartilage erosion. Although joints are the primary target of RA, extra-articular manifestations can have a significant impact on other organ systems. Patients may present with extra-articular features, including sub-cutaneous nodules, vasculitis, pericarditis and pulmonary fibrosis, especially in the more severe cases. This autoimmune disorder affects approximately 1% of the population worldwide, making it the most common form of inflammatory arthritis. The age of onset is typically between 25 and 50, in the midst of working life, with significant social and economic impact, although it can occur at any age. The course is variable, ranging from mild brief illness affecting a few joints with minimal damage, to a progressive polyarthritis that leads to pronounced functional impairment and deformity.

Conventional treatment choices for RA include corticosteroids and disease-modifying anti rheumatic drugs (DMARDs) and for patients who fail to respond adequately to these drugs the additional use of biopharmaceuticals, in particular Tumor necrosis Factor α (TNF-α)-inhibitors, offers greater opportunities for disease management. However, despite the undoubted success of anti-TNFα, about 40% of patients treated with a TNFα inhibitor do not respond (Wijbrands and Tak, 2017). In addition, up to 50% of primary responders lose their response within 12 months of the start of therapy (Buch et al., 2007; Juarez et al., 2012). Besides, these drugs do not specifically target the root cause of the disease. They interfere with generic steps in immune response thus they may be associated with systemic side effects, for example increased risk for infections (Wang et al., 2018; Liao et al., 2017; Atzeni et al., 2012). Considering that continuous, lifelong therapy for RA is required in most cases to relieve symptoms and prevent long-term joint damage,
and that patients on biologics are often on concomitant medications, such as steroids and/or DMARDs, the risk of serious infections and hospitalization could severely affect the quality of life of patients, especially in old age and if comorbidities are present (Goh et al., 2013). RA remains therefore a chronic condition for which there is currently no effective cure.

Lack of knowledge of the disease-specific human pathophysiology and etiology severely impedes development of targeted drugs for RA. This could partly be a consequence of the overuse of animal models that often cannot accurately recapitulate human RA etiopathogenesis and drug responses, and the inadequate consideration and/or use of human relevant research methods.

Animal models of arthritis have been used extensively to identify druggable targets for RA and test potential therapeutics. Despite they have been extremely useful to test new approaches of intervention, concerns about low clinical development success rates for investigational drugs (Hay et al., 2014; Hartung, 2013), coupled with increasing awareness of the ethical issues surrounding the use of animal models, have led many to question their utility in the study of complex human conditions and drug target identification. Although traditional human cell cultures have been invaluable in the study of pathogenesis of RA and for drug discovery research, excessively simplistic cellular models, often utilizing cancer cell lines or nonhuman cells cultured under non-homeostatic and non-physiologic in vitro conditions could have hampered, to some extent, the understanding of the pathogenetic mechanisms of RA, lacking behind in drug target identification. New emerging sophisticated cell culture systems and tools could increase our understanding of disease and improve our search for effective therapeutics, while reducing the number and replacing animals employed in biomedical research.

The need for a paradigm shift is becoming increasingly evident, as the limitations of traditional models are more and more recognized. In chemical toxicology a transformation is already unfolding, following a seminal report from the US National Research Council in 2007 (Krewski et al., 2010; NRC, 2007). This recommended a “21st-century paradigm” for safety testing, involving an explicit transition away from a reliance on adverse endpoints in animal tests and towards a novel framework based on understanding toxic perturbations to cellular pathways, mainly using in silico tools and human-specific cell and tissue models. Such transition is actively supported at European level, with the EU legislation governing animal experimentation (European Directive 2010/63/EU on the protection of animals used for scientific purposes) (SCHER, 2013), as well as by U.S. regulatory and research agencies both from environmental and medical arenas (Collins, 2011). Novel technologies are being integrated into toxicology and environmental health research and are also applicable to disease research (Langley et al., 2015). Although some techniques are still in their infancy and several challenges remain to be overcome, recent developments have brought about an amazing array of tools and research approaches that are offering bold new ways to study RA and could potentially yield profuse and meaningful human relevant data.

Another important fact is that RA is a multifactorial disease influenced by a number of known modifiable risk factors, such as smoking and food choices (Lahiri et al., 2012). Some of the factors identified already form part of the healthy lifestyle advice given for cardiovascular diseases and cancer prevention, and prevention of RA may be a bystander motivating factor in high-risk individuals, such as those with first-degree relatives with RA. Formalizing this into a focused prevention may be a highly cost-effective public health initiative.

Here, some of the major limitations associated with traditional in vivo and in vitro models of RA are discussed, along with the potential and limitations of human-based new approach methodologies. Finally, we also highlight the importance of prevention and the impact of environmental and life style factors in the risk of RA.

2 RA research: the inadequacy of conventional in vitro and in vivo models and current paradigms

So far, RA has predominantly been studied using a variety of in vitro assays and animal models. Cell-based in vitro assays are based on relatively simple (co)culture systems and assays generally used to study cell adhesion, migration, antigen presentation and lymphocyte activation (Pretzel et al., 2009; Giese and Marx, 2014). Cell and tissue cultures are invaluable tools in RA research, especially those of human origin. Traditional human synovial cultures have been crucial in the achievement of TNF-α blockers, to date the most successful drug to manage RA (Brennan et al., 1989). The therapeutic effect of TNF-α blockers were subsequently confirmed in an animal model (Keffer et al., 1991).

However, using cells and tissues under static and nonphysiologic condition (e.g., petri dishes), could severely affect the relevance of results. The absence of extracellular matrix and other cell types results in alterations of cell functions and a rapid loss of phenotype (Parnies and Hartung, 2017). In particular, the lack of physiological stimuli impairs the reliability of traditional static models. These stimuli can be classified into three major groups (Figure 1): (i) the biochemical signals from other cells and the extracellular matrix, (ii) the physical and structural stimuli from the three-dimensional (3D) microenvironment, and (iii) the mechanical stimuli derived from movement and the physicochemical fluxes originating from temperature, concentration or momentum gradients (Di Nardo et al., 2011).

Traditional in vitro methods present several shortcomings (Table 1) and lack clinical disease context. Therefore, it is becoming increasingly evident that more relevant and predictive in vitro models are needed to better simulate the aforementioned stimuli and increase understanding of RA pathological mechanisms.

On the other hand, the use of animal models for RA is intrinsically flawed for several reasons. Although there is general agreement among the scientific community that the immune systems of mammalian species show remarkable similarities (Ernst and Carvunis, 2018), human immune responses are markedly different from those of animals, especially rodents (Mestas and Hughes, 2004; Zschaler et al., 2014). A poignant example of this unmet need is the immunotherapy TGN1412 (targeting CD28 signalling), which caused an unpredicted cytokine storm in 6 patients (Stebbens et al., 2007; Hunig, 2012). In particular, Dayan and Wraith (2008) have identified several factors to explain TGN1412 failure, which are mainly related to poor prediction of risk based on preclinical studies, and the breadth of the margin of safety required both in dose and trial design when testing a treatment with a novel mode of action. This suggests that accuracy in study design and data interpretation may help mitigate or prevent possible translational failures. Nevertheless, several studies also suggest the existence of interspecies differences in CD28 signalling between human and animals, in particular mice and monkeys, (Schraen and Kalinke, 2008; Porciello et al., 2018; Waiblinger et al., 2008). Such differences, despite important and scientifically sound attempts to improve accuracy in study design, are reliably the major factors underlying failure and lack of translatability.
Fig. 1: The three axes of stimuli that act on cells, tissues, organs and organisms. The biochemical signals from other cells and the extracellular matrix, the structural stimuli from the three-dimensional (3D) microenvironment, and the mechanical stimuli derived from physicochemical fluxes originating from temperature, concentration or momentum gradients.

Tab. 1: Thirteen major shortcomings of static, one-dimensional cell culture models

| S1 | Nutrient and metabolite transport is limited by diffusion. |
| S2 | It is difficult to create and maintain controlled concentration gradients. |
| S3 | Extracellular concentrations in vitro mimic neither extracellular concentrations in vivo nor the relationship of these latter concentrations to intravascular concentrations. |
| S4 | Open-surface cultures may not have significant interstitial flow and the associated signaling. |
| S5 | It is hard to reverse experiments, i.e., achieve rapid washout without disrupting the cells. |
| S6 | Daily or less-frequent media changes result in significant cyclic changes in nutrients, metabolites, and pH. |
| S7 | It is not possible to provide shear forces to maintain endothelial and epithelial polarization. |
| S8 | It is difficult to provide mechanical forces to cells without the use of cumbersome, vacuum actuated, flexible-bottom chambers. |
| S9 | Small-volume wells with a supposedly homogeneous cellular phenotype do not recapitulate the heterogeneous tissue microenvironment. |
| S10 | The microenvironment in the corners at the outer circumference of a well in a plate may not reflect that at the centre of the well. |
| S11 | Wells near the outside of a plate may have different gas environment than those at the center. |
| S12 | It is difficult to create well-to-well connections with controlled flow that can model organ-organ interactions. |
| S13 | Centralized fluid handler and plate reader hardware are not well suited for:  
- Simultaneous dynamic experiments on a large number of different wells;  
- Fast, real-time, closed-loop control of the chemical and mechanical microenvironment;  
- Complex exposure protocols. |

As summarized by Davis, (2008), it has been proposed that mice are poorly representative of the human immune system, for three main reasons: (i) the use of inbred strains induce a prevalence of homozygous recessive defects that may skew the regulation of the immune response (von Herrath and Nepom, 2005); this may be extremely important considering the lethal effects induced by some heterozygous deletions of cytokines involved in phenotype development (Ferrara, 1999); (ii) animal models of humans disease are often carefully and arbitrarily planned according to a specific biologic or therapeutic purpose; this is the opposite of human disease, which serendipitously occurs as an independent variable and demands treatment to be tailored according to individual’s needs (Quintana-Murci et al., 2007); (iii) the million of years of evolutionary divergence among animals exposed to significantly different environmental challenges play a role that cannot be dismissed (Mestas and Hughes, 2004).

Dozens of preclinical arthritis models have been developed in a variety of species (e.g., mouse, rat, rabbit and monkey) that involve spontaneous or induced synovial inflammation. The most commonly utilized animal species in RA research is the mouse. Several murine models of arthritis have been established (Brand, 2005), including those that require immunization with antigen [proteoglycan-induced arthritis (P gia) (Finnegan et al., 1999), streptococcal cell-wall arthritis (Koga et al., 1985), collagen induced arthritis (CIA) (Courtenay et al., 1980) and antigen-induced arthritis (Brackertz et al., 1977)]; those induced by chemical agents [oil-induced arthritis (Hopkins et al., 1984); spontaneous models [tumor necrosis factor-α transgenic mouse (Butler et al., 1997) and K/BxN T-cell receptor transgenic mouse (Kouskoff et al., 1996)]; and humanized models (Schinnerling et al., 2019). While all these models exhibit some of the classical features found in RA, i.e., joint swelling, synovitis, pannus formation, and bone erosion, each model differs in speed of disease onset, chronicity, severity, resolution and histopathology (McNamee et al., 2015). The histopathology of the rodent models also differs between each other, as well as with human RA (Patel, 2010). None of these models is truly RA, and none consistently predicts the effect of a therapeutic agent in patients. For instance, interleukin-6 deficiency has little or no effect in passive transfer models of arthritis or in tumor necrosis factor transgenic mice, and methotrexate (to date the first-line DMARD for RA treatment), is only marginally effective in collagen-induced arthritis (CIA), which has been linked, by Delano et al., (2005), to genetically based resistance to methotrexate-induced anti-inflammatory effects in DBA/1 mice. Anti-CD20 antibodies (a next generation drug widely employed in RA) only work when administered very early in CIA, but not in established disease. For all these drugs, considering the preclinical (animal model) results, without clinical data, could have led investigators to abandon an effective therapeutic approach. Conversely, positive data in rodents might lead to overestimation of the therapeutic effect in humans; for example, nonsteroidal antiinflammatory drugs are remarkably effective in rat adjuvant arthritis, but provide only modest relief for RA patients (Hegen et al., 2008; Firestein, 2009). Soto et al. (2008) performed a gene array analysis comparison between rat CIA and human RA, to evaluate how closely the rat model reflects human RA. They concluded that while there are both similarities and
differences in the gene expression between human RA and rat CIA, the differences in gene expression profiles between the two were of greater significance, suggesting different inflammatory and pathogenetic mechanisms.

Furthermore, Seok et al. (Seok et al., 2013) show that, although acute inflammatory stressors from different etiologies result in highly similar genomic responses in humans, the responses in corresponding mouse models correlate poorly with the human conditions.

Apart from possible interspecies differences hampering animal data relevance to the human condition, poor interpretation of animal data and/or inaccuracy in study design can be amongst the critical factors underlying lack of reliability (and reproducibility) of in vivo models. An example is the use of anti-CD4 antibody for the treatment of RA patients. While anti-CD4 monoclonal antibodies induced long-lasting disease suppression in CIA animal models, their use in patients with RA had been disappointing, due to poor penetration in the synovial joint in quantity sufficient to suppress the disease and without severe side effects (i.e., peripheral blood lymphopenia) (Bugelski et al., 2000; Choy et al., 1998). However, it has also been shown that anti-CD4 depleting antibodies can suppress CIA when administered before (i.e., prophylactically), but not after development of arthritis (Goldschmidt et al., 1992; Koberza et al., 2014). These studies may, in part, explain why most anti-CD4 antibody treatments in human RA have failed.

Together with rapidly increasing knowledge on the complex functioning and dysfunctions of the human immune system, consensus has emerged on limitations inherent to even the most sophisticated animal models (Zenzewicz et al., 2010; Davis, 2008; Khanna and Burrows, 2011).

3 New technologies: opportunities for a human biology-based RA research

Advances in stem cell technology, microengineering, microfluidics, computing power, and respective multidisciplinary cooperation, enabled the development of new technologies and approaches, which were inaccessible until a few years ago.

These technologies include: 1. several human-based models focused on the use of patient-derived cells, such as patient-derived induced pluripotent stem cells (iPSCs) and their differentiated derivatives, 2. tissue engineering and advanced in vitro technologies (e.g., fluidic bioreactors, microphysiological systems, etc.), 3. epidemiology and multi-omics approaches (e.g., genomics, proteomics, transcriptomics, exposomics, etc.) resulting from overall analyses of biological samples by high-throughput analytical approaches and databases, and 4. computational analytical models.

Given the need to integrate the huge amount of incoming data, comprehensive multi-scale and systems biology approaches are becoming fundamentally important. These approaches must take into account all the different levels of biological complexity (including population, individual, organ/tissue, cellular, protein, and gene level), thereby allowing for the elucidation of disease-related adverse outcome pathways (AOPs) as already envisioned in toxicology and applicable to human health research and drug discovery (Langley et al., 2017; Langley et al., 2015; Herrmann et al., 2019).

3.1 Human induced pluripotent stem cells (iPSCs)

The advent of iPSCs and the reprogramming technology (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) has revolutionised many fields, notably those of disease modelling and cellular therapeutics (Avior et al., 2016). Importantly, such cells can self-renew for many cell divisions and can be differentiated into a broad range of different cell types. These characteristics enable the study of development and cellular function both in normal and disease states, and also allow large numbers of cells to be produced for high throughput genetic and drug screening, as well as cell therapy. Patient-derived iPSCs are of special interest where the isolation of primary human tissue is invasive and potentially harmful and therefore there is a limited availability of live cells and tissues. iPSCs have been derived from individuals with a variety of monogenic and polygenic disorders, including autoimmune diseases, and provide an invaluable resource for studying specific genetic contributions to human disease. iPSCs provide opportunities to capture the heterogeneity that arises from gender, ethnicity and gene modifiers specific to patients from which they have been obtained. Reprogrammed somatic cells from patients are already applied in disease modelling, drug testing and drug discovery, thus enabling researchers to undertake studies for treating diseases “in a dish” (Son et al., 2016; Bassett, 2017).

In particular, Lee et al. (2014a) reprogram fibroblast-like synoviocytes (FLSs) from patients with rheumatoid arthritis (RA) to generate disease-specific and patient-specific iPSCs. RA may be a promising target disease for iPSC applications because of its complex pathophysiology. Patient-specific iPSCs are in fact particularly useful for studying diseases with complex mechanisms, which are affected by several factors that range from the genetic background to environmental modifications (Lee et al., 2014a; Natsumoto et al., 2017).

To delineate the systemic pathophysiology of RA, the ideal approach would be to perform disease modelling using a patient’s own cells or tissues. However, the possibility of obtaining live cells and tissues could be very limited due to the fact that RA systemic inflammation often affects extra-articular sites, such as heart, lungs and gut. In these cases, tissue biopsy only secures a small number of target cells and involves the patient undergoing an invasive procedure implying ethical issues. Thus the acquisition of a patient’s own tissues is poorly applicable for research purposes.

Cardiovascular disease (CVD) is the most commonly encountered comorbidity and is the leading cause of mortality and morbidity in patients with RA (Avina-Zubieta et al., 2008; Avina-Zubieta et al., 2012; Avior et al., 2016).

Lee et al. (2016) successfully obtained functional cardiomyocytes from FLSs in RA patients through iPSCs reprogramming, paving the way for studying pathological implications of the disease in a human biology-based perspective. iPSCs were successfully employed to study several cardiological conditions and the effect of drugs on myocardium, and they have a great potential to study
RA-associated cardiological implications, providing insight into the pathogenetic mechanisms (Musunuru et al., 2018; Feric et al., 2019; Sala et al., 2019).

A growing number of researchers have employed iPSCs for modeling a variety of diseases and conditions, including neurological (Sanchez-Danes et al., 2012; Pistollato et al., 2014; Pamies et al., 2017) endothelial (Kurokawa et al., 2017; Cochrane et al., 2019), cardiovascular (Zhang et al., 2015b; Liang and Du, 2014), renal (Kim et al., 2018b), gastrointestinal (Takahashi et al., 2018), as well as autoimmune diseases (Tang et al., 2016; Iizuka-Koga et al., 2017; Son et al., 2016).

Additionally, advanced genome-editing technologies, such as the clustered regularly-interspaced short palindromic repeats/CRISPR-associated protein-9 nucleases (CRISPR/Cas9) can now be used to add, disrupt or modify the sequence of specific genes related to a given disease and measure their impact on human iPSC-derived cells (Bassett, 2017; Mungenast et al., 2016). In particular, these nucleases can induce guided DNA breaks, which can be repaired by homologous recombination with a donor vector carrying a desired point mutation or gene, in order to better model the disease in vitro (Byrne and Church, 2015; Hendriks et al., 2016).

A disease-associated gene mutation can be introduced into iPSCs, and the analysis of these cells can reveal the biological mechanisms of genetic susceptibility. Various iPSC lines derived from the same specimens should be considered, as this approach could reduce confounding variables. For example, genome-edited iPSCs could clarify the biological functions of disease-susceptibility genes in autoimmune diseases in the same genetic and environmental background (Shoda et al., 2018).

It is possible to culture and differentiate large numbers of iPSCs that can be used for drug screening. By differentiating iPSCs into immune cells (Choi et al., 2009; Yangagimachi et al., 2013; Senju et al., 2011), a system reflecting the pathogenesis of autoimmune diseases can be established, and this system could be a promising tool for screening drugs. Identification of novel drug discovery targets is expected in such a system (Shoda et al., 2018).

It could also be applied to drug toxicity testing. hiPSCs provide a unique opportunity to investigate the organ-specific and patient-specific toxic effects of antirheumatic drugs. They have already been used to successfully reproduce the long-term hepatotoxicity of Methotrexate in a human based setting (Kim et al., 2018a).

Despite the great potential of iPSCs, their broad applicability and reliability is currently hampered by some limitations. It is essential to clearly recognize these constraints and define strategies to overcome them. It is generally recognized that generating high quality iPSC lines is still expensive and time consuming. In addition, only a limited number of RA iPSC-derived lines have been generated and thoroughly characterized so far. Different programming and quality control methods are often employed, as well as a variety of somatic cell types. These differences in protocol make inter-laboratory comparisons difficult. Moreover, reprogramming is often based on the use of integrating lentiviruses and retroviruses, which may cause insertional mutagenesis that may alter the biology of the iPSCs and interfere with their differentiation into somatic cell types. Many approaches remain xenon-contaminated and stem cell culture systems that rely on undefined animal-derived components introduce variability to the cultures. For this reason, to minimize these issues, current and future reprogramming methods should aim to be xeno-free and based on the use of non integrating reprogramming vectors or entirely vector-free approaches. Given the peculiar nature of iPSCs, a high level of standardization of undifferentiated cell cultures as well as of the differentiation process is required in order to ensure the establishment of robust test and research systems (Pistollato et al., 2012).

Newly made iPSC lines should also be assessed for genomic integrity via cytogenetic karyotyping or array-based virtual karyotyping. The latter has the advantage of potentially detecting copy number changes and microdeletions and microduplications. There has in fact been concern that iPSCs can accumulate mutations during the process of reprogramming (Gore et al., 2011), which makes them less than perfectly matched to the donor individuals.

Another fact that should be taken into account when using iPSCs is the “epigenetic memory”. There is evidence that the epigenetic signatures of the somatic cells of origin might be retained in the reprogrammed iPSCs. Nevertheless, there is evidence that iPSCs lose epigenetic traits during long term culture (Nishino et al., 2011), which might be considered either as a positive aspect (as the epigenetic memory of somatic cells of origin might be mitigated) or a negative aspect (in light of the fact that RA patient epigenetic signatures might also be lost over time).

Nevertheless, the iPSCs approach holds enormous potentials to study RA pathogenesis and the rapidly expanding research field is already tackling these limitations.

3.2 Tissue engineering approaches

Tissue engineering (TE) was defined by Langer and Vacanti, (1993), in early 90s as “an interdisciplinary field which applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function”. TE aims to induce tissue-specific regeneration processes, thus overcoming the well-known drawbacks of organ transplantation (i.e., donor shortage, need of immunosuppressive therapy).

Although the most obvious application of tissue engineering is in regenerative medicine, TE approaches have been recently proposed for the design of reliable three –dimensional (3D) in vitro models of healthy or pathological tissues and organs, which can be employed for drug screening and the evaluation of new therapies, as well as the investigation of the complex phenomena regulating disease onset and progression.

Moving from the first definition of TE in the early 90s through the huge amount of work carried out and published by many research groups all over the world, researchers have gained high expertise in cell manipulation, materials science, and bioengineering for the design of highly complex biomimetic tissue substitutes for reparative/regenerative purposes. These tools and specific competences have been transferred in the last decade to the development of 3D engineered in vitro models.

RA is characterised by drastic thickening of the synovial membranes, followed by the formation of a proliferative synovial tissue (pannus) containing predominantly FLSs and neutrophils. The pannus tissue is responsible for the invasion and destruction of the underlying cartilage and bone (Doan and Massarotti, 2005; Andreas et al., 2008; Ibold et al., 2007).

Calvo et al. (2017) cultured patient-derived FLSs in 3D micromasses, challenged with TNF, to mimic synovial inflammation and study cellular mechanisms of pannus formation and inflammatory remodelling. When stimulated with TNF, hyperplasia resembling those observed in patients’ synovium, was detected. Gene expression studies revealed differentially expressed genes during the early phase and the mature phase of the culture period, allowing gaining insight into RA pathogenesis.
Damerau et al., (2019), developed a human-based *in vitro* 3D joint model to simulate the immune-mediated pathogenesis of RA. The model consists of different components including an osteogenic and chondrogenic part, the joint space with synovial fluid and the synovial membrane. It allows interactions between cells by signalling molecules and cell contacts. Human bone marrow derived mesenchymal stromal cells (hMSCs) were used to develop the different 3D tissue components. The arthritis joint was simulated by the application of neutrophils and typical cytokines. The authors confirmed and validated in a standardized manner phenotypic integrity and stability of each single component of the multi-component 3D *in vitro* joint model and have thus provided a suitable model to study the efficacy of drug treatments *in vitro* in a human based setting.

3D *in vitro* models give the possibility to independently identify and modulate cellular and molecular factors responsible for disease onset and progression, allowing the investigation of the contribution of each of them on the development of a specific disease and thus changing the way to study tissue physiology and pathophysiology. A 3D *in vitro* model allows the cells to grow and interact with each other and with the ECM in the all spatial dimensions. The introduction of these models in the RA research practice may lead to numerous advantages, such as the overcoming of the limits associated with traditionally employed models (i.e., animals and 2D cell culture models), and the achievement of more reproducible data, thanks to the possibility to tightly control the experimental parameters, reducing costs and time.

However, the biological complexity of 3D tissues entails high requirements regarding applicable culture techniques. For instance, ensuring a suitable nutrient supply throughout a 3D construct is challenging in comparison to 2D cell cultures (Alepee et al., 2014). Additionally, tissue-specific cues should mimic the *in vivo* situation to allow generating tissue constructs with required characteristics and functions (Schuerlein et al., 2017; Jin et al., 2015). New technologies such as Multicompartamental-modular Bioreactors (MCmB) and microphysiological systems could address these issues, allowing exposing cells and tissues to mechanical, biochemical or electrical stimuli, as well as fluidic perfusion.

### 3.3 Multicompartamental-modular Bioreactors (MCmBs)

As mentioned before, the complexity of the physiological environment is not replicated in petri dishes or microplates. All cells are exquisitely sensitive to their microenvironment, which is enriched with factors secreted by the surrounding cells and influenced by mechanical stimuli derived from flow, perfusion and movement. This is a major limitation to experiments investigating cellular responses *in vitro* since the complex interplay between mechanical and biochemical factors is generally missing.

A Multicompartamental-modular Bioreactor (MCmB) is an advanced interconnected cell culture flow system, engineered to provide *in vivo* like conditions for cell growth.

The MCmB is an innovative system for dynamic cell cultures and co-cultures. The modular chamber is designed with shape and dimensions similar to the 24-MultiWell, and consists of a cell culture chamber made of silicon polymer. The modular chambers can be also connected together in series or in parallel as desired, in order to replicate tissue/tissue and tissue/organs communications and recreate in vitro models of metabolism or diseases using the organomics approach.

The MCmB is able to apply controlled flow allowing a high medium flow rate and non turbulent fluid dynamics at the same time (Mazzei et al., 2010; Mattei et al., 2014).

Permeability studies across epithelial barriers are of primary importance in drug delivery, as well as in toxicology. However, traditional *in vitro* models do not adequately mimic the dynamic environment of physiological barriers.

The Membrane Bioreactor (MB) is a new double flow bioreactor for mimicking physiological barriers, which combines a transwell-like system with medium flow and multi-compartmental models. A porous membrane, whose characteristic and porosity may vary according to research needs, divides the bioreactor into two independent chambers for dynamic *in vitro* studies of drug diffusion through liquid–liquid or air-liquid physiological barriers (Giusti et al., 2014; Sbrana et al., 2013).

Sensorized Squeeze Pressure bioreactor is an innovative system for long-term cell culture and tissue engineering, able to apply a cyclic hydrodynamic and non-contact overpressure (the squeeze stimulus) using a simple vertical piston movement. This kind of stimulation is particularly useful for neo-tissues or fresh-constrasts seeded with articular chondrocytes, cardiomyocytes or endothelial cells. In fact, these cells require a dynamic environment to maintain their differentiate state, but at the same time do not tolerate direct compression or high shear stress (De Maria et al., 2011; Giusti et al., 2013).

Connected cultures of hepatocytes, adipocytes and endothelial cells in the MCmB have been used to investigate the regulation of systemic metabolism *in vitro*. The system has been designed using allometric scaling, focusing on glucose and lipid processing for their relevance to diabetes and metabolic disorders. Investigations on the role of adipose tissue and the effects of hyperglycemia, simulating diabetes type I and type II in the systemic model, have been reported (Iori et al., 2012).

MCmB have also been used to validate an *in vitro* model of small intestine. It has been demonstrated that the flow increases barrier integrity and tight junction expression of Caco-2 cells with respect to the static controls. Besides, the stimulus induced by flow increases transport across the barrier, closely mimicking the *in vivo* conditions (Giusti et al., 2014).

Further applications include cardiovascular stem cell differentiation (Pagliari et al., 2014), fluid shear stress on hepatocytes (Rashidi et al., 2016), an interconnected blood brain barrier model (Miranda-Azpiazu et al., 2018) and nanotoxicity with endothelial cells (Ucciferri et al., 2014).

The new generation fluidic bioreactors are equipped with integrated sensors and control systems. They are able to adjust environmental variables like pH, temperature, flow and hydrostatic pressure, in order to simulate the physiological environment and maintain the required parameters for long time (Mazzei, 2008; Giusti et al., 2017).

Mesenchymal stem cells are particularly suitable to maintain cell growth and but proliferation and some differentiation processes, they may not be suitable to model the anatomy of the affected joint, which is generally characterized by limited cell growth, hyperplasia, cell differentiation and cell death. Notwithstanding, with regards to RA research, MCmBs could offer a wide range of possibilities, from the investigation of articular pathogenetic mechanisms (e.g., the opportunity to set up a 3D dynamic arthritic joint model) to the study of the extra articular implications of RA, to the evaluation of the metabolism and toxic effects of anti rheumatic drugs.
3.4 Organ-on-chip / microphysiological systems

Organs-on-chips (OOC) are controlled microfluidic systems in which (human) cells are cultured in engineered microenvironments that recapitulate the essential aspects of tissue geometry, actuation, dynamics, flow and gradients found in the human body (Hu et al., 2011; Bhatia and Ingber, 2014). Microphysiological systems (MPS) consist of interacting OOC or tissue-engineered, 3D organ constructs that use human cells. Individually, each construct is designed to recapitulate the structure and function of a human organ or organ region, paying particular attention to the cellular microenvironment and cellular heterogeneity. When coupled together to create an MPS, these constructs provide the capability to analyze multiorgan interactions and offer the possibility of providing, in vitro, an unprecedented physiological accuracy for disease modelling and drug discovery, allowing to investigate cell-cell, drug-cell, drug-drug, and organ-drug interactions. The list of OOC is ever-expanding, a wide range of tissues and organ systems have been modelled, including heart (Zhang et al., 2015b), gut (Pocevicute and Ismagilov, 2019), liver (Knolvon and Tasoglu, 2016), blood vessels (de Graaf et al., 2019), a breathing and immune-reactive lung composed of human airway, capillary and immune cells (Hu, 2015), kidney (Lee and Kim, 2018) brain (Pamies et al., 2017; Mozaffazal Jahromi et al., 2019), lymphoid follicle (Goyal et al., 2018), spleen (Rigat-Brugarolas et al., 2014), bone marrow (Sieber et al., 2018) and complex MPS that connects engineered tissues from up to 10 organs (Edington et al., 2018) to simulate a human-body-on-a-chip. Human-on-a-chip refers to an in vitro model mimicking either normal or pathological whole human physiology within a microfluidic system that has high measurement accessibility and control. Ultimately, MPS could be used to create, with iPSC-derived cells, a human-on-a-chip tailored to a single patient for use in a personalized or precision medicine scenario (Wikswo, 2014). The concept of precision medicine, in which each individual would receive tailored treatment for the promotion, maintenance and restoration of their health, is becoming increasingly important in medicine, toxicology, pharmacology and biomedical science due to the increasing recognition of groups of non-responders. This current lack of “precision” in medicine contributes to inefficient healthcare in which many patients receive treatments that are not beneficial for them (Schork, 2015), and this is particularly true for RA (Romao et al., 2013; Strand et al., 2018).

There are a number of challenges related to 3D organ constructs and OOC, particularly when multiple organs are coupled together to create MPS to model drug-organ-organ interactions and organ-organ regulation. These include achieving a proper scale in terms of organ size and cell number, attaining architectural complexity of the human tissues and organs in vitro and in a miniaturized manner, developing a universal perfusion medium suitable for multiple cell types within the same organ or within different organs connected together, the need for small and controlled fluid volumes, accounting for the contributions of missing organs, organ vascularization and revision of culture protocols (Halldorsson et al., 2015; Park et al., 2019; Wikswo, 2014). Despite there are still several issues to overcome, the potential of these tools for research is considerable. Microfluidics-based chip technology is currently in a mature state and offers exceptional control over culture conditions along with other conditions, i.e., spatial homogeneity, chemical gradients, time-dependent biochemicals, substrate mechanical properties, etc. (Schwarz and Bischofs, 2005; Sosa-Hernandez et al., 2018; Weibel and Whitesides, 2006; Luni et al., 2010, 2014). Microfluidic OOC/MPS devices allow the analysis and use of less volume of samples, chemicals and reagents, reducing the global costs of applications. Another advantage of microfluidic OOC/MPS is the ability to incorporate analytical biosensors into the culture platform, thus combining living cells and sensors for detection of cellular physiological parameters and analysis of external stimuli in situ, in a non-invasive way (Halldorsson et al., 2015). In that regard, engineering approaches have been used to develop physical, chemical and biological sensors can be integrated to OOC. These sensors have been shown to provide reproducible results in a short time with data transmission, multiplexing and on-line monitoring capability by analysing very low volume of samples. Readout technologies are based on measurement of physical parameters associated with tissue/organoid microenvironment (such as O2, pH, CO2 and osmolarity), biological properties (protein and metabolite secretion, DNA methylation etc.), morphology (cell layer barrier, cell-cell interaction, via fluorescence and confocal microscopy) (Shanti et al., 2018).

The simultaneous electrochemical, mass spectrometric, and optical measurement of the dynamics of tens to hundreds or even thousands of cellular variables will allow an unprecedented advance in our understanding of living cells and how they respond to pharmaceuticals, pathogens, cellular or environmental stimuli. The emergence of new technologies has refined the MPS capability for translational research (Mittal et al., 2019), thus these systems have the potential to dramatically impact RA research, providing a wealth of opportunities to understand the RA pathogenesis and afford a potentially better model for drug discovery and screening, in particular with regard to the emerging area of personalized medicine. Although the pathophysiological processes, biomechanical and hydrodynamic pressure conditions characterizing RA in humans are still not fully elucidated, some studies, such as Gottardi (2019) are attempting to model over-physiologic compression forces in an osteoarthritis. The “human joint on a chip” is an example of how organ on chip technologies could be used to model joint diseases, including RA (Karperien, 2019).

Targeting human FLS migration and invasion-mediated bone erosion is a promising clinical strategy for the treatment of RA. Ma et al., (2018) designed a microfluidic chip-based, cell co-cultured platform to mimic RA FLS - mediated bone erosion and performed drug-sensitive assay. The migration and invasion to bone-related cells were reconstructed on a microfluidic model, providing an effective, human-based anti-RA drug screen model.

3.5 Epidemiological studies and novel “multi-omics” readouts

Epidemiological studies have been important in identifying RA-related risk factors. The primary risk factors for RA include genetic factors (MacGregor et al., 2000), female sex (Crowson et al., 2011), age > 35 (Deane et al., 2017), cigarette-smoking (Costenbader et al., 2006), nutritional patterns characterized by high intake of red meat and low polyunsaturated fatty acids (Di Giuseppe et al., 2014), obesity (Versini et al., 2014), low socioeconomic status (Chen et al., 2015), emotional trauma and distress (Yilmaz et al., 2017). Additionally, exposure to air pollution (Essouma and Noubiap, 2015), chemicals and pesticides (Lundberg et al., 1994; Parks et al., 2011), and the intake of metals (Irfan et al., 2017) have been described as possible risk factors. Environmental and lifestyle risk factors are known to play a pivotal role in the onset of pathologic changes underlying RA, which often appear many years before the symptomatic stages (Deane, 2014).

Knowledge of these risk factors may enable the discovery of early biomarkers of RA and the development of intervention strategies to prevent or delay RA onset or progression, avoid complications and gain important insight on RA pathogenesis.

In particular, analysis of either RA-patient advanced imaging readouts, RA patient-derived synovial fluid and blood samples has been proven essential to identify possible early biomarkers of RA (Table 2).
The interaction of the HLA-DRB1 shared epitope gene and cigarette-smoking exposition seems to play a major role in the development of anti-citrullinated protein antibody (ACPA)-positive rheumatoid arthritis (Too et al., 2012; Padyukov et al., 2004). Positivity to ACPA correlates with a persistent, erosive disease (Jilani and Mackworth-Young, 2015) and are associated with a higher TNF-α serum level (Thilagar et al., 2018). An increased expression of interferon (IFN) -responsive genes, the so-called IFN signature, has been reported in rheumatoid arthritis (RA); the description of IFN expression signatures (Thurlings et al., 2010; Raterman et al., 2012; de Jong et al., 2015), has led to extensive insights into the mechanisms of disease and the development of new therapies. Despite no strong epidemiological evidence exists to link one or more specific air pollution particles to RA, the incidence of RA was reported in rheumatoid arthritis (RA); the description of IFN expression signatures (Thurlings et al., 2010; Raterman et al., 2012; de Jong et al., 2015), has led to extensive insights into the mechanisms of disease and the development of new therapies. Despite no strong epidemiological evidence exists to link one or more specific air pollution particles to RA, the incidence of RA was found to be higher in urban areas. Living near air pollution emitters was associated with higher risks of developing RA and of producing RA-specific autoantibodies (Sigaux et al., 2019).

The start of the 21st century was characterized by rapid advances in high-throughput and high-content-technologies, bioinformatics, medical-science, biology, and genetics pertinent to epidemiology. Enhanced computing and data-storage capacity have been critical (Hiatt et al., 2013). The advent of genomics and genome-wide association studies (GWASs), for example, has played an important role in promoting the transformation of the practice of epidemiology. Epidemiologic science moved in the omics era a decade ago, with genomics, proteomics, epigenomics, metabolomics, and many other omics readouts. Novel omics approaches have enabled epidemiologists to quantify characteristics that were not previously within their reach and to investigate features that up to a few years ago were not even in their sight.

GWASs have been performed for many years and allowed to identify RA-risk-associated genes as well as genetic factors associated with various disease subphenotypes, including production and circulating levels of autoantibodies and joint destruction (van der Helm-van Mil et al., 2006; Soleimani et al., 2017). Recent studies involving transcriptomics readouts have revealed the molecular effects of TNF blockers in the peripheral blood (Oswald et al., 2015) or synovial tissues (Ducrèix et al., 2014) of patients with RA. Metabolomic approaches have significantly contributed to the advancement of RA research. The differences in metabolite abundance can be measured in low volumes of patients’ synovial fluid to aid diagnosis, distinguish among different types of arthritis and improve understanding of disease mechanisms (Anderson et al., 2018; Hugle et al., 2012; Carlson et al., 2019).

Omic technologies allow the recognition of patterns of disease at a pathway level, thereby, to reclassify systemic autoimmune diseases, including RA, and to develop new therapeutics from a personalized perspective. The use of omic readouts could allow the discovery of correlative patterns involving drugs not currently suspected to be of value in systemic autoimmune diseases (Teruel et al., 2016).

Individually, these technologies have contributed medical advances that have begun to enter clinical practice. However, each technology individually cannot capture the entire biological complexity of most human diseases. Integration of multiple technologies, referred to as “multi-omic approach or systems biology” has emerged as an approach to provide a more comprehensive view of biology and disease (Karczewski and Snyder, 2018). The integration of data from diverse omics readouts provides multi-faceted insight into the interrelation of these omics layers on disease processes, allowing the retrieval of comprehensive and holistic biological information. Although early therapeutic intervention can result in sustained, drug-free, disease remission in RA patients (Aterido et al., 2018; Tasaki et al., 2018; Romão et al., 2017; Lindstrom and Robinson, 2010). Recent advances in high-throughput single-cell technologies (Prosperio and Mahata, 2016) are rapidly changing the technological landscape of biological sciences and human immunology and now make it possible to measure the (epi)genomic, transcriptomic, or proteomic state of individual cells (Leonavicius et al., 2019). Single-cell technologies can dissect the genotypic and phenotypic heterogeneity of bulk

---

**Table 2. Summary of biomarkers possibly useful for RA detection and follow-up**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Localization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Plasma</td>
<td>(Grassi et al., 1998)</td>
</tr>
<tr>
<td>ESR</td>
<td>Plasma</td>
<td>(Silva et al., 2010)</td>
</tr>
<tr>
<td>RF</td>
<td>Serum</td>
<td>(Heidari et al., 2009b)</td>
</tr>
<tr>
<td>Anti-ccp</td>
<td>Serum</td>
<td>(Heidari et al., 2009a)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Plasma</td>
<td>(Costa et al., 2019)</td>
</tr>
<tr>
<td>IL-6, IL-10, IL-13</td>
<td>Serum, synovial fluid</td>
<td>(Hornum et al., 2017; Wang et al., 2012; Liao et al., 2004)</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Serum, synovial fluid</td>
<td>(Fawzy et al., 2016)</td>
</tr>
<tr>
<td>C5a, C5aR</td>
<td>Synovial fluid</td>
<td>(Hornum et al., 2017)</td>
</tr>
<tr>
<td>FCN-2</td>
<td>Serum</td>
<td>(Cheng et al., 2014)</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>Serum</td>
<td>(Symersen et al., 2008)</td>
</tr>
<tr>
<td>sCTX-I, uCTX-II</td>
<td>Serum, urine</td>
<td>(Symersen et al., 2010)</td>
</tr>
<tr>
<td>Juxta-articular osteoporosis</td>
<td>Finger and wrist joints</td>
<td>(Moon et al., 2013; Berglin et al., 2004; Eyre et al., 2012; Johansson et al., 2006)</td>
</tr>
<tr>
<td>HLA-DRB1 *0404 /0401; SNP PTPN22 rs2476601; PAD14 rs2240336; IL6R rs228145;</td>
<td>Genome - Genetic loci associated with susceptibility to RA</td>
<td>(Eyre et al., 2012; Johansson et al., 2006; Berglin et al., 2004)</td>
</tr>
</tbody>
</table>

Abbreviations: IL, interleukin; TNF-α, tumor necrosis factor α; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; RF, Rheumatoid factor; Anti-ccp, anti-cyclic-citrullinated-peptide antibodies; C5a, Complement fraction 5a; FCN-2, Ficolin-2; MMP-3, Matrix Metalloproteinase 3; sCTX-I, uCTX-II, C-terminal crosslinking telopeptide of type I collagen; uCTX-II, urinary C-terminal crosslinking telopeptide of type II collagen; SNP, Single Nucleotide Polymorphism; PAD14, Peptidyl arginine deiminase, type IV.
tissue, and promise to deepen our understanding of the underlying mechanisms governing both health and disease. Through modification and combination of single cell assays available for omics profiling, single-cell multi-omics approaches have been developed to simultaneously and comprehensively study not only the unique genotypic and phenotypic characteristics of single cells, but also the combined regulatory mechanisms evident only at single cell resolution (Chappell et al., 2018).

Latest advances in single-cell technologies offer an opportunity to expand our understanding of human immune system and to identify disease-associated cell subsets in human tissues at high resolution in an unbiased fashion (Villani et al., 2017; Stephenson et al., 2018; Wong et al., 2016; Papalexi and Satija, 2017). Single-cell omics technologies have already indicated roles for T peripheral helper cells (Rao et al., 2017) and HLA-DR+CD27− cytotoxic T cells (Fonseka et al., 2018) in RA pathogenesis, besides, they have identified a distinct subset of fibroblasts enriched in RA synovial tissue (Mizoguchi et al., 2018). With the advent of high-throughput technologies and high-content assays, a systems-oriented approach to biological sciences is emerging, which represents a shift from the classical reductionist approach.

The novel concept of the “exposome”, accounting for the totality of environmental exposures from gestation onward, is currently considered complementary to the genome in the study of disease etiology. In particular, among the possible triggers of the autoimmune process and in particular of RA, cigarette smoking is a well known risk factor (Costenbader et al., 2006; Costenbader and Karlson, 2006; Liu et al., 2019). The study of its effect at the gene expression level, by means of transcriptionic and epigenomic analyses, is a field of broad scientific interest (Cho et al., 2017). Svendsen et al. (2016), conducted an epigenome-wide association study (EWAS) to search for gene independent, differentially methylated DNA positions and regions associated with RA by studying monozygotic twin pairs discordant for RA. Smoking and anti-cyclic citrullinated peptide antibodies were included as covariates. Researchers identified several differentially methylated regions associated with RA, which may represent environmental effects or consequences of the disease and plausible biological pathways pertinent to the pathogenesis of RA.

The challenge is in the integration and interpretation of these complex multi-omics datasets. The integration of such diverse data types may be considered one of the key challenges of present-day bioinformatics, due to different data formats, high data dimensionality and need for data normalization (Pinu et al., 2019; Fondi and Lib, 2015). The continuous exponential growth in omics data require similar development in software solutions for handling this challenge. New bioinformatic tools and pipelines for the integration of data from different omics disciplines continue to emerge, and will support scientists to reliably interpret data in the context of biological processes. Integrative omics tools offer the chance to handle this challenge, but further investigations and coordinated efforts are required to boost this field (Dihazi et al., 2018).

### 3.6 Computational and analytical models

Over the past decade, there has been a paradigm shift in how clinical and experimental data are collected, processed and utilized. Bioinformatics, machine learning (Yari et al., 2019) and artificial intelligence, fuelled by breakthroughs in high-performance computing, data availability and algorithmic innovations, are paving the way to effective analyses of large, multi-dimensional collections of patient biological samples, histories, laboratory results, treatments and outcomes. ML and other computational approaches can suggest effective solutions for the unsettled issues arising from complex and heterogeneous diseases, such as rheumatic diseases (Kim and Tagkopoulos, 2018; Obermeyer and Emanuel, 2016; Heard et al., 2014). ML applications in multi-omics datasets were examined in detail in a series of recent reviews (Ching et al., 2018; Libbrecht and Noble, 2015; Kim and Tagkopoulos, 2018; Wainberg et al., 2018).

Singh et al. (2018), present a systematic effort to summarize current biological pathway knowledge concerning RA and are constructing a detailed, interactive molecular disease map and a large-scale dynamical computational model for the study of RA synovial fibroblasts’ emergent behaviour under different initial conditions specific to RA. The map could be used as a template for omics data visualization offering a first insight about the pathways affected in different experimental datasets.

Since computational models are fairly dependent on the data they are trained on or are called upon to analyze, no model, regardless of its sophistication, can create a useful analysis from low-quality data. As the results returned by computational models are based solely on the input data and represent existing knowledge, these models are valid within the same framework of that knowledge and their performance will degrade if they are not regularly updated using new, emerging, human-biology-based and human-relevant data. Moreover, development and adaptation of integrated software platforms are central to efficient and effective use of data and for predictive computational modeling (Ghosh et al., 2011).

### 4 Adverse Outcome Pathways (AOPs)

An Adverse Outcome Pathway (Feric et al., 2019) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or toxicological effect. AOPs have a common structure comprising exposure to the first molecular initiating event (e.g., a chemical binds to a cell receptor), intermediate steps and key events and an adverse outcome that (in toxicology) could for example be cancer, allergy or liver damage. The AOP concept was originally developed in the field of risk assessment for chemicals (Landesmann et al., 2013) and ecotoxicology (Ankley et al., 2010). AOPs have been described for skin sensitization, liver cholestasis, liver steatosis and fibrosis (Vinken et al., 2013; Willett, 2014; OECD, 2014). The new paradigm in toxicology could provide a template for modernising the disease modelling and drug discovery paradigm. Langley et al. (2015) suggest a new conceptual framework that repurposes the 21st-century transition underway in toxicology. The disease AOPs, like AOPs in toxicology, describes a chain of causally linked key events causing downstream effects at several biological levels and provide clear mechanistic rationales for diagnostic, preventative, and therapeutic interventions in the era of personalized medicine. The central steps will likely be similar, although the molecular initiating events will be more varied. For example, as well as chemical perturbations, infectious and genetic factors may initiate the disease process. By using an AOP conceptual framework it could be possible to gather existing knowledge about signalling pathways that are perturbed at the onset and during the consolidation of the disease, and to link genetic determinants, lifestyle and environmental factors with adverse health effects (Pistollato et al., 2015). Recently, an AOP approach for Alzheimer disease research has been proposed (Langley, 2014). Incorporating advanced scientific tools into a research framework emphasising pathways and networks in human-specific models could offer better progress towards...
understanding and treating diseases than the current emphasis on animal models. The disease AOP concept would provide a unified framework for describing relevant pathophysiological pathways and networks across multiple biological levels and for encompassing extrinsic and intrinsic causes. Describing these pathways and networks, along with anchoring molecular initiating events with adverse outcomes, the AOP framework would represent a significant advance over existing concepts that are often studied in isolation and biological pathways or networks that are invariably considered only at the molecular or cellular levels.

The disease AOP approach would better exploit advanced experimental and computational platforms for knowledge discovery, since the emergence of AOP networks will identify knowledge gaps and steer investigations accordingly. A commitment to build, curate, and disseminate a pathways framework within the biomedical research field would thus provide considerable impetus to base decisions on mechanistic understanding rather than empirical observation, as has been the case in toxicology (Langley et al., 2015). It is important that the overall physiopathological scenario does not become lost when using AOPs. AOPs are to be considered as open and flexible structures that should be continuously refined by feeding in old and new data, coming from a human based and human relevant approach (Vinken, 2016; Langley et al., 2015).

5 Discussion

While traditional animal and cell culture models of RA have been useful to elucidate some of the mechanisms underlying RA, the use of non-human (animal) models to mimic the complexity of the pathophysiological processes, biomechanical and hydrodynamic pressure conditions characterizing RA in patients may potentially be misleading, in light of the numerous interspecies differences characterizing e.g., chondrocytes biology (Schulze-Tanzil et al., 2009), articular cartilage (Athanasiou et al., 1991) and cartilage thickness (McLare et al., 2012). Here we described some new technologies, tools and approaches that could be employed in an integrated human-based framework suitable for investigating cellular and molecular mechanisms underlying RA pathology, and pharmacotherapeutics. To our knowledge, this is the first study discussing the applicability of human-based methods and models for RA research. Similarly, other studies have discussed the use of human-based approaches for other autoimmune diseases, (van de Stolpe and Kaufmann, 2015; Shoda et al., 2018; Shin et al., 2019; Rogal et al., 2019), taking into account the limitations of traditional (animal) models, and the availability of new technologies.

In recent years, the shift toward a new human-based paradigm has been advocated extensively in toxicology and regulatory testing (NRC, 2007), but also in other research fields, including autoimmune diseases (Langley, 2014; van der Worp et al., 2010; Mak et al., 2014; Begley and Ellis, 2012; Geerts, 2009; van de Stolpe and Kaufmann, 2015). The envisioned human-based framework will not only increase human relevance and translatability, but also contribute to the reduction and/or replacement of animals traditionally used in RA research. Nowadays, in light of the growing concern for the ethical justification of the use of animals in research, it is very important to consider not only the scientific dimensions, but also the ethical cost inherent in the use of living beings.

Several human cell/tissue advanced models and tools have been developed, spanning from patient-derived pluripotent stem cells, three-dimensional engineered tissues, fluidic bioreactors and the more complex joint on a chip. Human-based cellular and tissue models and high-throughput (omics) readouts, supported by epidemiology studies, represent the basis of a paradigm shift in RA research that will increase knowledge of the molecular mechanisms that are perturbed at the onset of the disease, helping define novel biomarkers for early detection, and establishing preventive and more precise, targeted treatment strategies.

It has to be said that in the proposed strategic framework, the use of patient-derived cellular models such as iPSCs, and the application of omics readouts – while tackling human relevance - would still constitute the lower level/scale of “wet lab” research. Therefore, large computational approaches together with large-scale epidemiological data sets represent the essential tools required to account for higher level/scale and to establish systemic correlations among signaling pathways, epigenomic and genomic perturbations, patients’ heterogeneity, and lifestyle components. In this regard, some comprehensive maps of signaling pathways and networks that are dysregulated in RA, are being provided (Singh et al., 2018; Ostaszewski et al., 2018; Wu et al., 2010). These maps may help in identifying genes as predictors of RA risk, in combination with other omics data. There is a growing understanding of risk factors that may be mechanistically related to RA development, including lifestyle factors, such as smoking and nutrition.

In particular, accumulating research evidence suggests that individual dietary patterns might be implicated in the risk of developing RA (Skoczyńska and Świękrot, 2018; Philippou and Nikiforou, 2018). Another aspect is the potential evidence on the pathophysiology of RA mediated through the gut microbiome. Human-based approaches (in vitro, in silico, etc.) should take into account also these aspects, including e.g., the microbiota in complex in vitro models (Jalili-Firoozinezhad et al., 2019), and/or accounting for nutrigenomics, which cannot be reliably studied in animal models considering the unavoidable interspecies differences. Although the relationships among diet, microbiota, and human health are complex, the new tools, such as the metagenome sequencing, provide new connections and insights (Tong, 2015; Zhang et al., 2015a). The study of the effect of nutrients at the gene expression level, through nutrigenomic analyses, have received increasing attention (van Riel and Renskers, 2016; Higgins et al., 2018), advanced imaging (Vyas et al., 2016; Gu et al., 2011), the analysis of patient-derived synovial fluid- and plasma-related biomarkers, together with computational models and high-throughput readouts applied to patient-derived cell-based models to assess signaling pathways, post-translational, translational, and transcriptional events, represent an invaluable and more reliable strategy to better understand RA pathology, predict long-term sequelae, and develop successful

Discussion

While traditional animal and cell culture models of RA have been useful to elucidate some of the mechanisms underlying RA, the use of non-human (animal) models to mimic the complexity of the pathophysiological processes, biomechanical and hydrodynamic pressure conditions characterizing RA in patients may potentially be misleading, in light of the numerous interspecies differences characterizing e.g., chondrocytes biology (Schulze-Tanzil et al., 2009), articular cartilage (Athanasiou et al., 1991) and cartilage thickness (McLare et al., 2012). Here we described some new technologies, tools and approaches that could be employed in an integrated human-based framework suitable for investigating cellular and molecular mechanisms underlying RA pathology, and pharmacotherapeutics. To our knowledge, this is the first study discussing the applicability of human-based methods and models for RA research. Similarly, other studies have discussed the use of human-based approaches for other autoimmune diseases, (van de Stolpe and Kaufmann, 2015; Shoda et al., 2018; Shin et al., 2019; Rogal et al., 2019), taking into account the limitations of traditional (animal) models, and the availability of new technologies.

In recent years, the shift toward a new human-based paradigm has been advocated extensively in toxicology and regulatory testing (NRC, 2007), but also in other research fields, including autoimmune diseases (Langley, 2014; van der Worp et al., 2010; Mak et al., 2014; Begley and Ellis, 2012; Geerts, 2009; van de Stolpe and Kaufmann, 2015). The envisioned human-based framework will not only increase human relevance and translatability, but also contribute to the reduction and/or replacement of animals traditionally used in RA research. Nowadays, in light of the growing concern for the ethical justification of the use of animals in research, it is very important to consider not only the scientific dimensions, but also the ethical cost inherent in the use of living beings.

Several human cell/tissue advanced models and tools have been developed, spanning from patient-derived pluripotent stem cells, three-dimensional engineered tissues, fluidic bioreactors and the more complex joint on a chip. Human-based cellular and tissue models and high-throughput (omics) readouts, supported by epidemiology studies, represent the basis of a paradigm shift in RA research that will increase knowledge of the molecular mechanisms that are perturbed at the onset of the disease, helping define novel biomarkers for early detection, and establishing preventive and more precise, targeted treatment strategies.

It has to be said that in the proposed strategic framework, the use of patient-derived cellular models such as iPSCs, and the application of omics readouts – while tackling human relevance - would still constitute the lower level/scale of “wet lab” research. Therefore, large computational approaches together with large-scale epidemiological data sets represent the essential tools required to account for higher level/scale and to establish systemic correlations among signaling pathways, epigenomic and genomic perturbations, patients’ heterogeneity, and lifestyle components. In this regard, some comprehensive maps of signaling pathways and networks that are dysregulated in RA, are being provided (Singh et al., 2018; Ostaszewski et al., 2018; Wu et al., 2010). These maps may help in identifying genes as predictors of RA risk, in combination with other omics data. There is a growing understanding of risk factors that may be mechanistically related to RA development, including lifestyle factors, such as smoking and nutrition.

In particular, accumulating research evidence suggests that individual dietary patterns might be implicated in the risk of developing RA (Skoczyńska and Świękrot, 2018; Philippou and Nikiforou, 2018). Another aspect is the potential evidence on the pathophysiology of RA mediated through the gut microbiome. Human-based approaches (in vitro, in silico, etc.) should take into account also these aspects, including e.g., the microbiota in complex in vitro models (Jalili-Firoozinezhad et al., 2019), and/or accounting for nutrigenomics, which cannot be reliably studied in animal models considering the unavoidable interspecies differences. Although the relationships among diet, microbiota, and human health are complex, the new tools, such as the metagenome sequencing, provide new connections and insights (Tong, 2015; Zhang et al., 2015a). The study of the effect of nutrients at the gene expression level, through nutrigenomic analyses, have received increasing attention (Rana et al., 2016; van Breda et al., 2015; Ferguson et al., 2016; Ferguson, 2013) and could help in establishing a prevention strategy for RA.

In addition, the discovery of elevations disease-related biomarkers prior to the onset of clinically apparent RA raises hopes that individuals who are at risk for future RA can be identified in a preclinical phase of disease that is defined as abnormalities of RA-related immune activity prior to the clinically apparent onset of joint disease. Taken as a whole, these findings suggest that it may be possible to use biomarkers and other factors to accurately identify the likelihood and timing of onset of future RA, and intervene with lifestyle risk factor modification to prevent the future onset of RA in at-risk individuals.

Moreover, the impact of prevention on RA is pivotal. Behavioral and lifestyle factors can importantly affect both the prevalence and severity of RA (Donzelli and Schivalocchi, 2016). Large scale epidemiological and interventional studies are needed to improve our understanding of lifestyle related factors associated with the risk of RA and design effective prevention strategies.

Combining data derived from a wide range of studies, also accounting for the Disease Activity Score (DAS) and hand grip test (van Riel and Renskers, 2016; Higgins et al., 2018), advanced imaging (Vyas et al., 2016; Gu et al., 2011), the analysis of patient-derived synovial fluid- and plasma-related biomarkers, together with computational models and high-throughput readouts applied to patient-derived cell-based models to assess signaling pathways, post-translational, translational, and transcriptional events, represent an invaluable and more reliable strategy to better understand RA pathology, predict long-term sequelae, and develop successful
treatments. The envisioned framework will help redefine human RA pathogenesis and etiology according to a more holistic perspective, taking into account the numerous human-related risk factors implicated in the onset and consolidation of RA (Figure 2).

The generally reductionist approach followed in biomedical research might hamper the discovery of effective treatments for RA. According to Stanich and colleagues (2009), focusing research on single etiologic agents or factors possibly involved in RA pathogenesis, has been and continues being misleading. The authors discussed a number of factors relating directly or indirectly to the etiology and pathogenesis of RA, with the intention to demonstrate that previous studies of infectious, genetic, or other causation taken in isolation have provided little or no useful insight into that etiology. They suggest that when the existing data are holistically taken in context and as a whole, a picture does emerge as to how RA pathology is initiated. The picture indicates that, contrary to current research paradigms, RA should not be considered as a discrete clinical entity with a single, unique etiological source, but rather as a complex multifactorial syndrome, a common endpoint for a number of different starting points (Firestein, 2014). Since many factors contribute to RA etiology, and they do so differentially in individual patients, modern research must take these multifactorial aspects into account.

The feasibility of the envisioned human-based strategy necessarily requires the combined application of several methods and readouts and, eventually, of multiple areas of expertise and laboratory facilities. The establishment of a collaborative strategy is clearly imperative to determine what occurs throughout the course of RA. Increasing the awareness of the limits of traditional approaches, together with the perception of the potential advantages associated with 21st century human-relevant scientific approaches, is important to overcome the resistance to change (Tralau et al., 2012; Archibald et al., 2015).

6 Conclusion

Advanced human-based cellular models, high-throughput (omics) readouts, computational models, together with data obtained from meta-analysis of epidemiological and interventional studies, are among the ideal tools to unravel etiopathological aspects of RA in a human-based milieu and to predict environment-elicited biological perturbations occurring in RA, accounting for multiple levels of complexity, from population/individual scale down to gene level. We direct our review to scientists, teachers, advocates, funders and institutions who are involved or interested in RA research.

References


Butler, D. M., Malfait, A. M., Mason, L. J. et al. (1997). Dba1 mice expressing the human tnf alpha transgene develop a severe, erosive arthritis: Characterization of the cytokine cascade and cellular(281,757),(376,788)


https://www.researchgate.net/publication/263617666_Bioreactor_for_dynamic_biological_barriers


Wu, Y. L., Savelli, S. L., Yang, Y. et al. (2007). Sensitive and specific real-time polymerase chain reaction assays to accurately determine copy number variations (cnvs) of human complement c4a, c4b, c4-long, c4-short, and rccx modules: Elucidation of c4 cnvs in 50 consanguineous subjects with defined hla genotypes. J Immunol 179, 3012-3025. doi:10.4049/jimmunol.179.5.3012


**Conflict of interest**
The authors have no conflicts of interest to declare.