



Review Article

Rheumatoid Arthritis Research in the 21st Century: Limitations of Traditional Models, New Technologies, and Opportunities for a Human Biology-Based Approach

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease characterized by progressive bone and cartilage destruction, functional impairment, and long-term disability. Although RA has been described in the medical literature for over two hundred years, its etiology and pathophysiology are insufficiently understood. The current treatment of RA is mainly empirical or based on drugs that interfere with generic steps of the 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 approaches 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 discuss novel tools applicable to design human-oriented RA research, while fostering the need for a more holistic and preventative approach to the disease. Our goal is to stimulate 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 joint inflammation, and various degrees of bone and cartilage erosion. Although joints are the primary target of RA, extra-articular manifestations, including sub-cutaneous nodules, vasculitis, pericarditis, and pulmonary fibrosis, can have a significant impact on other organ systems. 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 a brief, mild 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). For patients who fail to respond adequately to these drugs, the additional use of biopharmaceuticals, in particular inhibitors of

Received October 1, 2019; Accepted November 27, 2019; Epub December 17, 2019; © The Authors, 2019.

ALTEX 37(2), 223-242. doi:10.14573/altex.1910011

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tumor necrosis factor α (TNF α), offers opportunities for disease management. However, despite the undoubted success of anti-TNF α treatment, about 40% of patients are non-responders (Wijbrandts 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 cause of the disease but interfere with generic steps of the immune response; thus they may be associated with systemic side effects, for example an increased risk of infection (Wang et al., 2018; Liao et al., 2017; Atzeni et al., 2012). As continuous, lifelong therapy for RA is required in most cases to relieve symptoms and prevent long-term joint damage, and because patients on biopharmaceuticals are often on concomitant medication such as steroids and/or DMARDs, the risk of serious infections and hospitalization can severely affect the quality of life of patients, especially in old age and if comorbidities are present (Goh et al., 2013). RA therefore remains a chronic condition for which there is currently no effective cure.

Lack of knowledge of the disease-specific human pathophysiology and etiology of RA severely impedes the development of targeted drugs. 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 *in vitro* research methods. Animal models of arthritis have been used extensively to identify druggable targets for RA and test potential therapeutics. Despite their having been extremely useful to test new approaches of intervention in many cases, 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 the pathogenesis of RA and for drug discovery research, simplistic cellular models, often utilizing cancer cell lines or nonhuman cells cultured under non-homeostatic and non-physiologic *in vitro* conditions are limited in their ability to fully reflect the pathogenetic mechanisms of RA. Emerging sophisticated human cell culture systems and tools promise to increase our understanding of RA and improve our search for effective therapeutics, while reducing 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 numerous research areas. This shift started in chemical toxicology following a seminal report from the US National Research Council (NRC, 2007; Krewski et al., 2010), which recommended a “21st-century paradigm” for safety testing, involving an explicit transition away from 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. This transition is actively supported at European level in form of the EU legislation governing animal experimentation (Directive 2010/63/EU) (SCHER, 2013), as well as by U.S. regulatory and research agencies both from environmental and medical are-

nas (Collins, 2011). The novel technologies being integrated into toxicology and environmental health research are also applicable to disease research (Langley et al., 2015). Although some techniques are still at early stages and several challenges remain to be overcome, recent developments have brought about an amazing array of tools and research approaches that offer bold new ways to study RA and could potentially yield profuse and meaningful human relevant data.

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 (NAMs). Finally, we highlight the importance of prevention and the impact of environmental and life style factors on the risk of RA.

2 The inadequacy of conventional *in vitro* and *in vivo* models and current paradigms

So far, RA has 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 used to study cell adhesion, migration, antigen presentation, and lymphocyte activation (Pretzel et al., 2009; Giese and Marx, 2014). Traditional human synovial cultures were crucial in the development of TNF α blockers, to date the most successful drug to manage RA (Brennan et al., 1989), before the therapeutic effect was confirmed in an animal model (Keffer et al., 1991).

However, we now know that traditional cell culture does not provide the physiological stimuli needed to maintain cell function and phenotype. These stimuli can be classified into three major groups (Fig. 1): (i) biochemical signals from other cells and the extracellular matrix, (ii) physical and structural stimuli from the three-dimensional (3D) microenvironment, and (iii) mechanical stimuli derived from movement and the physicochemical fluxes originating from temperature, concentration or momentum gradients (Di Nardo et al., 2011; Pamies and Hartung, 2017). Traditional *in vitro* methods thus present several shortcomings (Tab. 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 thus better model 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 in many respects (Ernst and Carvunis, 2018), human immune responses are still markedly different from those of rodents (Mes-

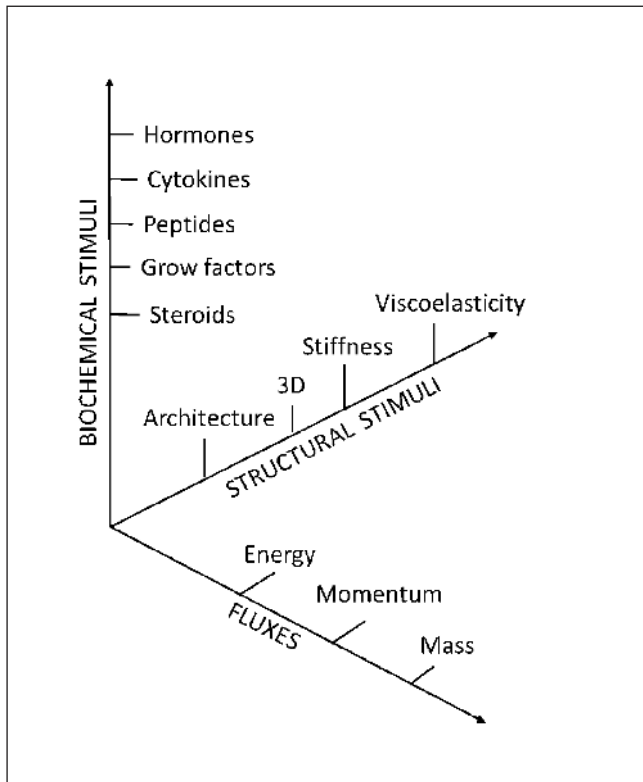


Fig. 1: The three axes of stimuli that act on cells, tissues, organs and organisms

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.

tas and Hughes, 2004; Zschaler et al., 2014). A poignant example of this issue was the immunotherapy trial of TGN1412 (targeting CD28 signaling), which caused an unpredicted cytokine storm in six volunteers (Stebbins et al., 2007; Hunig, 2012). Dayan and Wraith (2008) identified several factors to explain TGN1412 failure, mainly related to poor prediction of risk based on outcomes of preclinical studies and the narrow margin of safety required both in dose and trial design when testing a treatment with a novel mode of action. The poor prediction of risk is thought to stem from interspecies differences in CD28 signaling between humans and other animals, in particular mice and monkeys (Schraven and Kalinke, 2008; Porciello et al., 2018; Waibler et al., 2008).

As summarized by Davis, (2008), the murine immune system poorly represents the human immune system for three main reasons: (i) the use of inbred strains is associated with a prevalence of homozygous recessive defects that may skew the regulation of the immune response (von Herrath and Nepom, 2005); this may be highly relevant considering the lethal effects induced by some heterozygous deletions of cytokines involved in phenotype development (Ferrara, 1999); (ii) animal models of human 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 to the individual's needs (Quintana-Murci et al., 2007); (iii) the millions of years of evolutionary divergence of animals exposed to significantly different environmental challenges (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 induced by immunization with antigen (proteoglycan-induced arthritis (PGIA) (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 these models all 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 among the models and from human RA (Patel, 2010).

None of the animal models is truly human RA, and none consistently predicts the effect of a therapeutic agent in human patients. For instance, IL-6 deficiency has little or no effect in passive transfer models of arthritis or in TNF 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) results without any clinical data could have led investigators to abandon an effective therapeutic approach. Conversely, positive data in rodents may lead to overestimation of a therapeutic effect in humans; for example, nonsteroidal anti-inflammatory drugs are remarkably effective in rat adjuvant arthritis, but provide only modest relief to RA patients (Hegen et al., 2008; Firestein, 2009). Soto et al. (2008) performed a gene array comparison between rat CIA and human RA to evaluate how closely the rat model reflects human RA. Although there were similarities between human RA and rat CIA, the differences in gene expression profiles between the two were found to be of greater significance, suggesting different inflammatory and pathogenetic mechanisms. Furthermore, Seok et al. (2013) showed that, although acute inflammatory stressors from different etiologies result in highly similar genomic responses in different humans, the responses in corresponding mouse models correlate poorly with those of humans.

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

S1	Nutrient and metabolite transport are limited by diffusion.
S2	It is difficult to create and maintain controlled concentration gradients.
S3	Extracellular concentrations <i>in vitro</i> mimic neither extracellular concentrations <i>in vivo</i> 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 differ from that at the center of the well.
S11	Wells near the outside of a plate may have a 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: <ul style="list-style-type: none"> – 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.

Apart from possible interspecies differences hampering the relevance of animal data to the human condition, poor animal study design or data interpretation can be among the critical factors underlying the 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 was disappointing, due to poor penetration into the synovial joint in quantities 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; Kobezda 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 of the human immune system, consensus has emerged on the limitations inherent to even the most sophisticated animal models (Zenewicz et al., 2010; Davis, 2008; Khanna and Burrows, 2011). The development of autoimmune diseases, including RA, is influenced by complex underlying genetics, including the many genetic variations determining individual immune system performance (Feuk et al., 2006; Wu et al., 2007; Seok et al., 2013; Orru et al., 2013; Lee et al., 2014b). Altogether, this suggests that while animal models may have been useful to elucidate some of the mechanisms of arthritis,

they are not suited to develop novel targeted treatments for RA. Even mice with a humanized immune system lack the complex human genetics underlying autoimmune diseases like RA, preventing modeling of the human immune response and tolerance mechanisms (Shultz et al., 2012).

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

Promising new technologies and approaches that are relevant for RA research include: i) 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, ii) tissue engineering and advanced *in vitro* technologies (e.g., fluidic bioreactors, microphysiological systems, etc.), iii) 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 iv) computational analytical models.

Given the need to integrate huge amounts of incoming data, comprehensive multi-scale and systems biology approaches are becoming fundamentally important. These approaches must take into account all levels of biological complexity (including population, individual, organ/tissue, cellular, protein, and gene level) to allow the elucidation of disease-related adverse outcome pathways (AOPs) as already formalized in toxicology and

applicable to human health research and drug discovery (Langley et al., 2015, 2017; Herrmann et al., 2019).

3.1 Human induced pluripotent stem cells (hiPSCs)

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 is limited as systemic RA 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.

The advent of human iPSCs and stem cell reprogramming technology (Takahashi and Yamanaka, 2006; Takahashi et al., 2007) has revolutionized disease modelling and cellular therapeutics (Avior et al., 2016). These cells can self-renew for many cell divisions and can be differentiated into a broad range of different cell types, enabling the study of development and cellular function, both in normal and disease states, or allowing large numbers of cells to be produced for high throughput genetic and drug screening or for cell therapy. iPSCs have been derived from individuals with a variety of monogenic and polygenic disorders, including autoimmune diseases, and provide an invaluable resource for studying genetic contributions to human disease. iPSCs also 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 drug testing, drug discovery (Son et al., 2016; Bassett, 2017) and 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).

Patient-derived iPSCs are of special interest in diseases of complex pathophysiology where the isolation of primary human tissue is invasive and potentially harmful such as in RA (Lee et al., 2014a; Natsumoto et al., 2017). Lee et al. (2014a) have reprogrammed fibroblast-like synoviocytes (FLSs) from patients with RA to generate disease-specific and patient-specific iPSCs and also RA-patient derived functional cardiomyocytes (2016) as cardiovascular disease is the most commonly encountered comorbidity and is the leading cause of mortality and morbidity in patients with RA (Avina-Zubieta et al., 2008, 2012; Avior et al., 2016). iPSCs have been successfully employed to study several cardiological conditions and the effect of drugs on myocardium, and they have 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).

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). Using different iPSC lines derived from the same specimens (same genetic and environmental background), genome-edited iPSCs could be used to clarify the biological functions of disease-susceptibility genes in autoimmune diseases such as RA (Shoda et al., 2018).

Generation and differentiation of large numbers of iPSCs, e.g., into immune cells (Choi et al., 2009; Yanagimachi et al., 2013; Senju et al., 2011) reflecting the pathogenesis of RA, could be suitable for drug screening (Shoda et al., 2018). hiPSCs also 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. Generating high-quality iPSC lines is still expensive and time consuming. Only a limited number of RA-specific iPSC-derived lines have been generated and thoroughly characterized so far. These have been derived from a variety of somatic cell types using different programming and quality control methods, which can 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 in turn alter the biology of the iPSCs and interfere with their differentiation into somatic cell types. For these reasons, 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. 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 be assessed for genomic integrity via cytogenetic karyotyping or array-based virtual karyotyping. The latter can detect copy number changes, and microdeletions and microduplications. There has also been concern that iPSCs can accumulate mutations during the process of reprogramming (Gore et al., 2011).

There is evidence that the epigenetic signatures of the somatic cells of origin may be retained in reprogrammed iPSCs. However, it appears 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).

In all, the iPSC approach holds enormous potential to study RA pathogenesis and the rapidly expanding research field is already tackling its limitations.



3.2 Tissue engineering approaches

Tissue engineering (TE) was defined by Langer and Vacanti, (1993) in the 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 primarily aims to induce tissue-specific regeneration processes, thus overcoming the well-known drawbacks of organ transplantation (i.e., donor shortage, need of immunosuppressive therapy). However, TE approaches have been recently harnessed for the design of three-dimensional (3D) *in vitro* models of healthy or pathological tissues and organs for drug screening and the evaluation of new therapies as well as for investigation of the complex phenomena regulating disease onset and progression.

RA is characterized 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 and challenged them with TNF to mimic synovial inflammation and study cellular mechanisms of pannus formation and inflammatory remodeling. TNF challenge induced hyperplasia resembling that observed in patient synovium. Gene expression studies revealed differentially expressed genes during the early phase and the mature phase of the culture period, shedding light on RA pathogenesis.

Damerou et al. (2019), developed a human-based *in vitro* 3D joint model to simulate the immune-mediated pathogenesis of RA. The model consists of an osteogenic and chondrogenic part, the joint space with synovial fluid, and the synovial membrane. It allows interactions between cells via signaling molecules and cell contacts. Human bone marrow-derived mesenchymal stromal cells (hMSCs) were used to develop the different 3D tissue components. The arthritic joint was simulated by the application of neutrophils and typical cytokines. The authors confirmed and validated in a standardized manner the 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 allow the independent identification and modulation of cellular and molecular factors responsible for disease onset and progression, allowing investigation of their contributions to disease development. The cells can grow and interact with each other and with the ECM in all spatial dimensions. These models allow overcoming the limits of traditionally employed models (i.e., animals and 2D cell culture models) and promise more reproducible data by tightly controlling experimental parameters, reducing costs and time. However, the biological complexity of 3D tissues entails complex requirements of culture techniques. For instance, ensuring a suitable nutrient supply throughout a 3D construct is more challenging than for 2D cell cultures (Alepee et al., 2014). Additionally, tissue-specific cues are needed to mimic the *in vivo* situation and allow generation of tissue constructs with the required characteristics and

functions (Schuerlein et al., 2017; Jin et al., 2015). New technologies such as multicompartmental-modular bioreactors (MCmB) and microphysiological systems (see below) could address these issues by allowing exposure of cells and tissues to mechanical, biochemical or electrical stimuli, as well as fluidic perfusion.

3.3 Multicompartmental modular bioreactors (MCmBs)

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.

An MCmB is an advanced interconnected cell culture flow system engineered to provide *in vivo*-like conditions for cell growth of 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 connected in series or in parallel in order to replicate tissue/tissue or tissue/organ communication and to recreate *in vitro* models of metabolism or disease using the organomics approach. The MCmB can apply controlled flow, allowing a high medium flow rate with non-turbulent fluid dynamics (Mazzei et al., 2010; Mattei et al., 2014).

The Membrane Bioreactor is a double-flow bioreactor for mimicking physiological barriers, which combines a transwell-like system with medium flow and multi-compartmental models. A porous membrane, whose characteristics and porosity may vary according to research needs, divides the bioreactor into two independent chambers for dynamic *in vitro* studies of drug diffusion through physiological liquid-liquid or air-liquid barriers (Giusti et al., 2014; Sbrana et al., 2013). The Sensorized Squeeze Pressure bioreactor is a system for long-term cell culture and tissue engineering, able to apply a cyclic hydrodynamic and non-contact overpressure (the squeeze stimulus) using vertical piston movement. This stimulation is particularly useful for neo-tissues or fresh constructs seeded with articular chondrocytes, cardiomyocytes or endothelial cells, which require a dynamic environment to maintain their state of differentiation but do not tolerate direct compression or high shear stress (De Maria et al., 2011; Giusti et al., 2013). 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 over a long time (Mazzei, 2008; Giusti et al., 2017).

While bioreactor systems are particularly suitable to maintain cell growth and some differentiation processes, they may not be suitable to model the anatomy of the affected joint in RA, which is generally characterized by limited cell growth, hyperplasia, cell differentiation and cell death. However, MCmBs could offer possibilities to investigate articular pathogenetic mechanisms

(e.g., a 3D dynamic arthritic joint model), to study the extra-articular implications of RA, and to evaluate the metabolism and toxic effects of anti-rheumatic drugs.

3.4 Organ-on-chip / microphysiological systems

Organs-on-chip (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 (Huh et al., 2011; Bhatia and Ingber, 2014). Microphysiological systems (MPS) consist of interacting OOC or tissue-engineered 3D human organ constructs. 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 allow the analysis of multiorgan interactions and allow disease modelling and drug discovery *in vitro* with an unprecedented physiological accuracy, including the investigation of cell-cell, drug-cell, drug-drug, and organ-drug interactions. A wide range of tissues and organ systems have been modelled, including heart (Zhang et al., 2015b), gut (Pocevicicute and Ismagilov, 2019), liver (Knowlton and Tasoglu, 2016), blood vessels (de Graaf et al., 2019), a breathing and immune-reactive lung composed of human airway, capillary and immune cells (Huh, 2015), kidney (Lee and Kim, 2018), brain (Pamies et al., 2017; Mofazzal 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 connect engineered tissues from up to 10 organs (Edington et al., 2018) to simulate a human-on-a-chip.

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 (Wikswow, 2014). The concept of precision medicine, in which each individual would receive tailored treatment for the promotion, maintenance and restoration of their health, is gaining interest due to the increasing recognition of groups of non-responders. This current lack of tailored 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).

A number of challenges are related to 3D organ constructs and OOC, particularly when multiple organs are coupled together 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; Wikswow, 2014).

Microfluidics-based chip technology is currently at a mature stage and offers exceptional control over culture conditions, i.e., spatial homogeneity, chemical gradients, time-dependent

biochemical stimulations, 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 low volumes of samples, chemicals and reagents, reducing the costs of application. Analytical biosensors can be incorporated into the culture platform, thus allowing the detection of cellular physiological parameters and analysis of external stimuli *in situ* in a non-invasive way (Halldorsson et al., 2015). These sensors have been shown to provide reproducible results in a short time with data transmission, multiplexing and on-line monitoring ability by analyzing very low volumes 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.), and 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, and 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 RA pathogenesis and affording a potentially better model for drug discovery and screening, in particular with regard to the emerging area of personalized medicine.

Ma et al. (2018) have designed a microfluidic chip-based co-culture platform to mimic RA FLS-mediated bone erosion, providing an effective, human-based anti-RA drug screening model. 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), and Gottardi (2019) is attempting to model over-physiologic compression forces in osteoarthritis on a chip.

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), and emotional traumas and distress (Yilmaz et al., 2017). Additionally, exposure to air pollution (Essouma and Noubiap, 2015; Sigaux et al., 2019), 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 play a pivotal role in the onset of pathologic changes underlying RA, which often appear many years before 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 into RA pathogenesis.

In particular, analysis of RA-patient advanced imaging readouts, synovial fluid, and blood samples has identified possible early biomarkers of RA (Tab. 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 RA (Too et al., 2012; Padyukov et al., 2004). Positivity to ACPA correlates with a persistent, erosive disease (Jilani and Mackworth-Young, 2015) and is associated with a higher TNF α serum level (Thilagar et al., 2018). Also, an increased expression of interferon (IFN)-responding genes, the so-called IFN signature, has been reported in 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.

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. Omics technologies allow the recognition of patterns of disease at a pathway level and, thereby, to reclassify systemic autoimmune diseases, including RA, and to develop new therapeutics from a personalized perspective. The use of omics 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). For example, genome-wide association studies (GWASs) have identified 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), transcriptomics readouts have revealed the molecular effects of TNF blockers in the peripheral blood (Oswald et al., 2015) or synovial tissues (Ducreux et al., 2014) of patients with RA, and metabolomic approaches have been employed to aid diagnosis, distinguish among different types of arthritis, and improve understanding of disease mechanisms (Anderson et al., 2018; Hogle et al., 2012; Carlson et al., 2019). Individually, these technologies have contributed medical advances that have begun to enter clinical practice. However, each technology by itself cannot capture the entire biological complexity of human disease. Integration of multiple technologies, referred to as a “multi-omics” approach or “systems biology” has emerged 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 (Ajeganova and Huiz-

Tab. 2: Summary of biomarkers possibly useful for RA detection and follow-up

Biomarkers	Localization	References
CRP	Plasma	Grassi et al., 1998
ESR	Plasma	Silva et al., 2010
RF	Serum	Heidari et al., 2009b
Anti-ccp	Serum	Heidari et al., 2009a
TNF α	Plasma	Costa et al., 2019
IL-6, IL-10, IL-13 IL-15, IL-17, IL1 β	Serum, synovial fluid	Hornum et al., 2017; Wang et al., 2012; Liao et al., 2004
MMP-3	Serum, synovial fluid	Fawzy et al., 2016
C5a, C5aR	Synovial fluid	Hornum et al., 2017
FCN-2	Serum	Cheng et al., 2014
Eotaxin	Serum	Syversen et al., 2008
sCTX-I, uCTX-II	Serum, urine	Syversen et al., 2010
Juxta-articular osteoporosis	Finger and wrist joints	Moon et al., 2013; Berglin et al., 2004; Eyre et al., 2012; Johansson et al., 2006
HLA-DRB1 *0404 /0401; SNP PTPN22 rs2476601; PADI4 rs2240336; IL6R rs2228145;	Genome - Genetic loci associated with susceptibility to RA	Eyre et al., 2012; Johansson et al., 2006; Berglin et al., 2004

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, seric C-terminal crosslinking telopeptide of type I collagen; uCTX-II, urinary C-terminal crosslinking telopeptide of type II collagen; SNP, single nucleotide polymorphism; PADI4, peptidyl arginine deiminase, type IV

inga, 2017; Nagy and van Vollenhoven, 2015), it requires early diagnosis. Since current diagnostic tests are not sufficiently accurate or sensitive in the early stages of the disease, RA is typically diagnosed only once damage to the joints has already begun and when the window for optimal treatment may have been missed. The multi-omics approach has the potential to identify multiple biomarkers that can be used to transform the management of RA by enabling early diagnosis. Besides, through the analysis of biomarkers in patient populations, the disease could be stratified into distinct subsets that exhibit differential outcomes and responses to specific therapeutics or interventions (Aterido et al., 2018; Tasaki et al., 2018; Romão et al., 2017; Lindstrom and Robinson, 2010).

Recent advances in high-throughput single-cell technologies (Proserpio and Mahata, 2016) now make it possible to measure the (epi)genomic, transcriptomic, or proteomic state of individual cells at high resolution in an unbiased fashion (Villani et al., 2017; Stephenson et al., 2018; Wong et al., 2016; Papalexi and Satija, 2017; Leonavicius et al., 2019), and even allow the study of combined regulatory mechanisms evident only at single cell resolution (Chappell et al., 2018). Single-cell omics technologies have already indicated roles for peripheral T helper cells (Rao et al., 2017) and HLA-DR⁺CD27⁻ cytotoxic T cells (Fonseka et al., 2018) in RA pathogenesis and 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 transcriptomic and epigenomic analyses, is a field of broad scientific interest (Cho et al., 2017). Svendsen et al. (2016) identified gene-independent, differentially methylated DNA positions and regions associated with RA in monozygotic twin pairs discordant for RA by an epigenome-wide association study (EWAS) with smoking and anti-cyclic citrullinated peptide antibodies included as covariates. These regions may represent environmental effects or consequences of the disease and plausible biological pathways pertinent to the pathogenesis of RA.

The challenge lies in the integration and interpretation of these complex multi-omics datasets, which is hampered by different data formats, high data dimensionality, and the need for data normalization (Pinu et al., 2019; Fondi and Liò, 2015). The exponential growth of omics data requires a similarly fast development of software solutions to handle this challenge. New bioinformatics tools and pipelines for the integration of data from different omics disciplines continue to emerge to support scientists in reliably interpreting data in the context of biological processes (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 and machine learning (ML) fueled by breakthroughs in algorithmic innovations, high-performance computing, and data availability, 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 solutions for the issues arising from analyzing data on 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 behavior under different initial conditions specific to RA. The map could be used as a template for omics data visualization, offering a first insight into the pathways affected in different experimental datasets.

Since computational models are dependent on the data they are trained on or are called upon to analyze, their value depends on the quality of the data, they are valid only within the same framework of that knowledge, and their performance will degrade if they are not regularly updated. Development and adaptation of integrated software platforms is central to efficient and effective use of data and for predictive computational modeling (Ghosh et al., 2011).

4 Adverse outcome pathways (AOPs)

The AOP concept was originally developed in the field of risk assessment for chemicals (Landesmann et al., 2013) and ecotoxicology (Ankley et al., 2010). An AOP (Feric et al., 2019) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization 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 binding a cell receptor), intermediate key events, and an adverse outcome. AOPs have been described for skin sensitization, liver cholestasis, liver steatosis and fibrosis (Vinken et al., 2013; Willett, 2014; OECD, 2014).

This new paradigm in toxicology could provide a template for modernizing the disease modelling and drug discovery paradigm (Langley et al., 2015). A disease AOP, like an AOP in toxicology, describes a chain of causally linked key events causing downstream effects at several biological levels and provides 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 may 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 signaling 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). An AOP approach already has been proposed for Alzheimer disease (Langley, 2014).

Incorporating advanced scientific tools into a research framework emphasizing 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, such as disease mechanisms 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 pathophysiological 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

Animal and simple cell culture models of RA have been useful to elucidate some of the mechanisms underlying the disease. However, simple cell culture models cannot mimic the complexity of the pathophysiological processes, biomechanical and hydrodynamic pressure conditions characterizing RA, and animal models may be misleading owing to interspecies differences regarding e.g., chondrocyte biology (Schulze-Tanzil et al., 2009), articular cartilage (Athanasίου et al., 1991) and cartilage thickness (McLure et al., 2012).

Here we describe new technologies, tools and approaches that could be employed in an integrated human-based framework to investigate cellular and molecular mechanisms underlying RA pathology and pharmacotherapeutics. While other studies have discussed the use of human-based approaches for other autoimmune diseases (van de Stolpe and Kauffmann, 2015; Shoda et al., 2018; Shin et al., 2019; Rogal et al., 2019), this is, to our knowledge, the first review discussing the applicability of hu-

man-based methods and models for RA research taking into account the limitations of traditional (animal) models and the opportunities offered by new technologies.

In recent years, a 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 Kauffmann, 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 used in RA research. 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 patient-derived iPSCs, 3D 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 on the molecular mechanisms that are perturbed at the onset of the disease, helping to define novel biomarkers for early detection and establishing preventive and more precise targeted treatment strategies.

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. Large computational approaches together with large-scale epidemiological datasets represent the essential tools required to account for higher level/scale and to establish systemic correlations among signaling pathways, epigenomic and genomic perturbations, patient heterogeneity, and lifestyle components. Some comprehensive maps of signaling pathways and networks that are dysregulated in RA already have been compiled (Singh et al., 2018; Ostaszewski et al., 2018; Wu et al., 2010), which, in combination with other omics data, may help to identify genes as predictors of RA risk.

There is already a growing understanding how risk factors, including lifestyle factors, such as smoking and nutrition, may be mechanistically related to RA development. In particular, accumulating research evidence suggests that individual dietary patterns may be implicated in the risk of developing RA (Skoczyńska and Świerkot, 2018; Philippou and Nikiphorou, 2018). Another aspect is the growing insight into modulation of the pathophysiology of RA by the gut microbiome. Human-based approaches (*in vitro*, *in silico*, etc.) should also take into account these aspects, e.g. by including microbiota in complex *in vitro* models (Jalili-Firoozinezhad et al., 2019) and/or accounting for nutrigenomics, which cannot be reliably studied in animal models owing to interspecies differences. Although the relationships among diet, microbiota, and human health are complex, new tools, such as 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, has received increasing attention (Rana et al.,

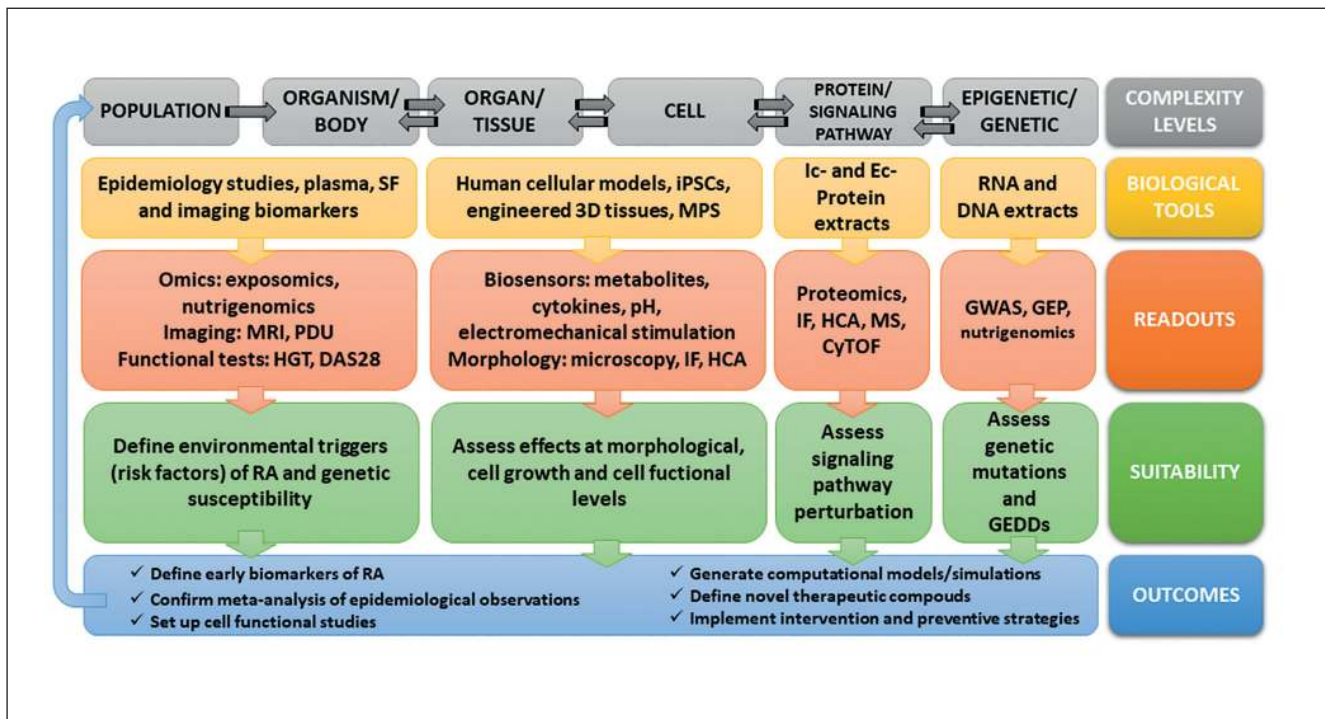


Fig. 2: Overview of the novel, available tools and readouts applicable to design human-based RA research, accounting for multiple levels of complexity, from molecular to population level

SF, synovial fluid; MRI, magnetic resonance imaging; PDU, power doppler ultrasonography; MPS, microphysiological systems; DAS 28, disease activity score-28; iPSCs, induced pluripotent stem cells; IF, immunofluorescence; HCA, high content analysis; MS, mass spectrometry; MRS, magnetic resonance spectroscopy; GWAS, genome-wide association studies; GEP, gene expression profiling; Ic and Ec, intracellular and extracellular; HGT, hand grip test; CyTOF, mass cytometry; GEDDs, gene expression dysregulations

2016; van Breda et al., 2015; Ferguson et al., 2016, 2013) and could help to establish a prevention strategy for RA.

The discovery of biomarkers that are elevated prior to the onset of clinically apparent RA would allow the identification of individuals who are at risk of developing RA in a preclinical phase of disease that is defined as “abnormalities of RA-related immune activity” prior to clinically apparent joint disease. This could make it possible to identify the likelihood and timing of onset of future RA and intervene with lifestyle risk factor modification to prevent the onset 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, will help us to better understand RA pathology, predict long-term sequelae, and develop successful treatments. The en-

visioned 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 (Fig. 2).

The reductionist approach traditionally followed in biomedical research may 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 is misleading: RA should not be considered a discrete clinical entity with a single, unique etiological source, but rather viewed 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 technology and expertise. 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 as well as of the advantages of 21st century human-relevant scientific approaches is essential to overcome resistance to change (Tralau et al., 2012; Archibald et al., 2015).



6 Conclusion

Advanced human-based cellular models, high-throughput (omics) readouts, and computational models, together with data obtained from the meta-analysis of epidemiological and interventional studies, are promising 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.

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Conflict of interest

The authors have no conflicts of interest to declare.