

# EXAMINATION OF CELL-HOST-BIOMATERIAL INTERACTIONS VIA HIGH-THROUGHPUT TECHNOLOGIES: A RE-APPRAISAL

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## **Abstract**

Biomaterials are required to act harmoniously when exposed to the body or bodily fluids. Investigating cellular or *in vivo* phenotypic responses and protein adsorption to the material surface helps to determine the associated biocompatibility. Past limitations on progress in this field include time-consuming cell-based screening tools and a limited understanding of the complex nature of cell–biomaterial interactions. While high-throughput technologies by their nature are a rapid tool to derive meaning from multifaceted systems and, in recent years, the biomaterial community is beginning to take advantage of these technologies, the key observation in this Leading Opinion Paper is that the biomaterials community has been slow to accept these methods as an addition to their traditional experimentation workflow. The purpose of this paper is to review the definition and recent usage of high-throughput experiments in order to examine biomaterial interactions at the cellular and wider host level, especially as they become more relevant within the biomaterials arena encapsulating tissue engineering, gene, drug and stem cell delivery systems. The technologies under focus include rapid cell-based screening, transcriptomics and proteomics.

**Keywords:** Biocompatibility; Transcriptomics; DNA microarrays; Proteomics; Mass spectrometry; Cell interaction

## 1. Introduction

The word ‘throughput’ appears to have two meanings; one is the rate of production or the time/speed at which data is received, and the second is the output relative to input or the amount of data transmitted between two points. On several occasions, the term ‘automated’ has also been added into the definition where the processes are only ever semi-automated with sometimes considerable user pre- and post-processing required [1]. When the term ‘high-throughput’ is applied to experimental technologies, the former definition holds true when considering classical molecular biology approaches. However, the second definition is becoming more applicable with the continuous improvement in the quantity and quality of data output in such methods. For our purposes, a high-throughput technology is “*a semi-automated process whereby, for any given input and the limitations set by the technology in question, the coverage and quantity of data output is comprehensive, normally thousands to millions of data points with a rapid processing speed*”.

High-throughput technologies have been applied in the fields of cancer research [2], [3] and [4], antiviral research in the characterisation of the host-pathogen dynamic [5], tissue engineering [6], genomic mutation rates [7], cardiovascular research [8], functional identification of the human genome [9], among many other disciplines. In 2006, we evaluated the application of -omic technologies in understanding cell-biomaterial interactions, where we predicted a shift in focus towards increased use of high-throughput methodologies [10]. Since then, the area has progressed only marginally, requiring a re-evaluation of our original projections.

High-throughput technologies have not had the same impact on the biomaterials field in comparison high-throughput and biomaterials to other disciplines. To exemplify, together the terms “high-throughput” and “biomaterials” appear approximately  $\sim 210$  times in PubMed [11] which is  $\sim 5\%$  of the hits returned when biomaterials is replaced with cancer. Why is this? Is the phenotypic response enough when deciding biomaterial success? First, let us consider what makes a biomaterial successful – biocompatibility.

Biocompatibility is the use or design of a material harmless to the host tissue, achieved either with chemical and biological inertness or successful integration with the surrounding proteins and cells. A material that is considered biocompatible will

have extensive applications in human health care, which could improve a patient's quality of life and elongate life expectancy. Existing biomaterials include joint replacements, artificial ligaments and tendons, heart valves, dental implants and contact lenses [12], [13], [14], [15] and [16]. Considering the average life expectancy of a human is now more than twice that from a century ago, we require more sophisticated medical tools with which to mend or aid our ageing bodies. The scientific field of biomaterials has improved significantly since one of the oldest recorded applications during wound healing; the use of linen sutures by the Egyptians over 32,000 years ago [17]. Subsequently, while we have continuously learned from the rejection and failure of biomaterials due to infection, inflammation and toxicological effects, complications can still occur [18], [19] and [20]. As such, accurate and reputable biocompatibility testing is an essential step for the detection or improvement of host-material responses.

Surface-adsorbed proteins play a key role, impacting upon the cellular responses and deciding the level of success of the biomaterial [21]. Ideally, a biomaterial should cause no adverse effects to the surrounding cells or tissues, similar to the "*First, do no harm*" or "*primum nil nocere*" as stated as one of the principal guidelines in medical ethics. Protein adsorption to a biomaterial surface is influenced by the surface properties, such as hydrophobicity, charge and chemistry, and is a dynamic event involving both adsorption and desorption [22], [23] and [24]. These characteristics also determine whether proteins are adsorbed in a biologically active conformation [25]. In most cases, successfully adsorbed proteins will allow for cell adhesion to the biomaterial through interactions with the protein layer, while unsuccessfully adsorbed proteins or proteins exhibiting an anti-adhesive effect will not permit cell adhesion and the material can be considered 'bioinert' [26]. Cell phenotype, including cell spreading, proliferation and morphology, are also affected by even discrete modifications of biomaterials, resulting in altered molecular responses. Ultimately, it is this cellular interaction with the biomaterial that will determine the biocompatibility of the implanted material. However, accompanying phenotype, perhaps the altered molecular environment could readily infer the potential of a biomaterial. This is discussed with several specific examples in Section 2. Nevertheless, as the focus in biomaterials moves away from inert biomaterials towards tissue engineering, stem cell and gene delivery systems, it becomes ever more necessary to comprehensively

assess the specific interactions between the cell/host and particular biomaterials of interest [27] (Fig. 1).

For example, tissue engineering over the last ~20 years has become a major focus of biomaterial sciences [28], [29] and [30]. The potential to regenerate and differentiate stem cells for clinical applications will benefit medicine significantly in the future. Problems to date with stem cell regeneration have been greatly overcome by the development of biomaterials that provide structural support for the growing cells and also in their ability to incorporate signals that promote desired cell functions. High-throughput technologies in this context have been used in several modern settings. These include the identification of key regulatory elements associated with the fibrotic response in order to optimize a tissue engineering strategy [31], understanding the environment ready to undergo tissue engineering [32] and defining a gene pattern signature [33]. Indeed, the move towards “smart” or active biomaterials requires a change in the way compatibility is monitored.

### **1.1. The evaluation of biocompatibility: a brief history**

Originally, histology was used to search for quantitative differences between cell types before and after cellular interaction with a biomaterial. Histological investigations suffer several limitations. The primary challenge is one of tissue preservation, as fixation and tissue dehydration will affect the phenotype of the cells, possibly altering their interpretation. Other challenges include staining variability and the subjective opinions of the pathologist that scores the stained tissue sections [34]. Notably, some of these issues have been overcome by the uniformity of tissue microarrays (TMA) [35]. Improvements also came in the early 1990s with the aid of computerised image analysis tools based on immunohistochemical (IHC) staining with monoclonal antibodies specific for a subset specific cell types, originally inflammatory cells [36] and [37]. More recently, automated image analysis software programs facilitate objective interpretation by enabling slide enhancement and quantification (see [34] for a thorough review) and, with continuous improvements, will reduce the immediate pressure on a pathologist whereby only a smaller percentage of scores may need verification. While IHC and image analysis are still widely used techniques, new methods are required for the purposes of examining

global cell–biomaterial interactions, which will encapsulate a more comprehensive picture of altered molecular responses and pathways in cells and tissues.

Northern blot analysis and reverse transcription polymerase chain reaction (RT-PCR) assays are used to study mRNA expression through the isolation and profiling of a specific mRNA (or discrete number of transcripts) in a sample. These techniques are useful for targeted experiments; for example, they have been used to assess the relationship between transforming growth factor-beta 1 (TGF- $\beta$ 1) and the mineralisation of an *in vitro* osteoblast/implant culture system [38]. In this case, both mRNA detection methods were carried out as Northern blotting was not sensitive enough to detect osteocalcin mRNA, one of the monitored ECM factors. While sensitivity can also be an issue using high-throughput methods such as microarrays an all-inclusive inspection of the transcriptome would provide information useful in cases where the investigators are looking at the physiological changes made through interactions of cells with biomaterials in terms of biocompatibility. Exploring the molecular environment and the genes which regulate this communication could help in early response prediction and in profiling fundamental positive and negative responses.

To this end, high-throughput expression studies have been used to characterise global cellular and molecular changes of biomaterial implantation (Table 1). These contemporary strategies are beginning to improve our understanding and gain significant insight into the nature of cell–biomaterial interaction. Importantly, we can start to address the questions of what pathways or networks are altered within the system under study and to what extent. One of the traditional, main weaknesses of biomaterials is their limited life-span, for example, the problem of tissue deterioration limiting the life-span of bioprosthetic valves to 10–15 years [39] and [40]. Notably, the introduction of mechanical valves has improved the longevity of synthetic valves to >25 years [40]. However, while these valves remain as a transient replacement, there also remains room for improvement. Thus, probing the genetic profile of a cell–biomaterial interaction over its lifetime and harnessing the capabilities of modern tools, one could permit the discovery of ordinarily imperceptible but consequential transformations. Transcriptomics and proteomics technologies enable the study of both the surface-adsorbed proteins, and the proteins expressed from the subsequent cell signalling cascades that occur in response to cell–biomaterial interaction. The

large quantity of data derived from these studies enables the investigator to identify previously unconsidered physiological alterations. Biocompatibility can also be region- and host-specific, meaning that testing across multiple sites and different organisms can result in distinct responses. This may be intuitively unexpected but is attributed to wide patient-to-patient variability [27] and lends further rationale for high-throughput tools to gain answers by studying the molecular environment as a whole and in a rapid, systematic manner.

## **2. High-throughput technologies**

### **2.1. Large-scale cell-based screening**

High-throughput screening (HTS) has been traditionally used within the pharmaceutical industry to rapidly examine a large collection of compounds in order to identify 'hits' in an *in vitro* assay, with the assay usually performed robotically in microtitre plates. Hit identification, in this context, is followed by lead optimisation of the compounds prior to administering to *in vivo* systems. The choice of cell types and assay is of paramount importance and should be selected with care. This will give rise to the most appropriate physiological read-outs that can be translated from an *in vitro* to an *in vivo* environment. In this respect, a range of assays are currently being developed that will allow more biologically useful information on the effects of compounds with cells. These assays aim to define the effects of compounds on cell viability, proliferation, differentiation, morphology and biocompatibility for *in vivo* studies. Ramirez et al. [41] recently reviewed cell viability and toxicity assays and outlined the variations and limitations of the assays *in vitro*. It is proposed that high-throughput platforms should be used regularly to predict the toxicology of drug candidates accurately. Blackmore et al. [42] used high content analysis (HCA) methods to screen 743 plasmids encoding developmentally regulated genes while functionally assessing neurite outgrowth. Changes in gene expression are hypothesised to be the reason why neurons in the central nervous system lose their capability to regenerate axons as they mature. This study is among the first examples of a high-throughput overexpression screen in post-natal CNS neurons. Daub et al. [43] have added the complexity of live-cell imaging of primary neurons to their high

content screen, allowing for real-time phenotypic cellular changes to be captured. Static features previously imaged are replaced with dynamic physiological live-cell images.

Such large-scale cell-based screening studies are starting to be applied in the biomaterials field [44]. It is widely known that there are limitations to the current tests used to study the effects of biomaterials on biological responses. The traditional assays that are used to test biological activities can be interfered with by the introduction of biomaterial. In order to overcome this problem and enable greater reproducibility, reliability and comparability of studies, it is becoming necessary to develop new types of assays and/or validate existing assays. Anderson et al. [45] utilised a high-throughput cell-based screening assay to evaluate a library of 2,350 structurally unique, degradable, cationic polymers to identify 46 that can transfect cells with a higher efficiency than conventional delivery systems. Similarly, Alder et al. [46] used a systematic approach to rapidly screen a panel of cell lines (SP2/0 myeloma, CHO, 1929 fibroblasts and J774 macrophages) treated with biomaterials that have previously been studied as carriers for drugs, proteins and vaccines. In this case, no appreciable cytotoxic effects were found on any of the four cell lines.

HTS and HCS technology has allowed for rapid, low-cost screening of biomaterials and has provided a platform to analyse multiple parameters including the effects on phenotypic and molecular properties of cells [47]. Nonetheless, there are limitations that could perhaps be said to lie mainly with the user and their choice in assay. Are the outputs of these assays sufficient for a comprehensive test of the full nature of cell–biomaterial interaction? Certainly, the visual effects, such as cell viability and morphology, are an excellent initial screen to decide the value and potential of a biomaterial. However, positive results from HTS/HCS assays do not purport faultless cell–biomaterial interactions and users need to be aware of the parameters or prospective changes that remain unaddressed.

## 2.2. Expression studies

### 2.2.1. Application of microarray technology to biomaterials

DNA microarrays have become standard, powerful tools to investigate global gene expression patterns. Microarrays have been used to investigate the changing environments in response to different types of biomaterials including metals, titanium, polymers and textile/fibrin gels. In between the first published study that availed of gene expression-oriented microarray technology to study cell–biomaterial interactions (carried out by Xynos et al. [48]), and our previous review [10], major insights into the transcriptomic responses affecting an array of cellular activities have been characterised. These responses extend to genes encoding ECM proteins [49], [50] and [51], cellular adhesion molecules [51] and [52] and proteins involved in inflammation [53] and apoptosis [48]. More recently, and as further elaborated below, the use of microarrays to profile the transcriptome in the context of biomaterial interactions, has continued to provide extensive molecular insights. This information, for example, the search for toxicological and cytotoxicological effects of distinct materials in relation to cells/organisms, could be harnessed for translation towards superior materials. The detection of only annotated transcripts, in the main, is one of the biggest drawbacks with DNA microarray technology, along with technical variation, cost and the complexity of statistical analysis involved.

#### 2.2.1.1. Use of microarrays in assessing the response of cells to metal ions

Both oligonucleotide and cDNA microarrays have been applied to studies aiming to reveal molecular mechanisms underlying cell–biomaterial interactions. In some cases, additional quantitative information, such as from real-time PCR, has verified these results. In addition, bioinformatics tools to contextualise these findings have uncovered important biological information including affected pathways and gene ontologies.

The biodistribution of gold nanoparticles (AuNPs) was the focus of a study by Balasubramanian et al. [54]. AuNPs in bulk are chemically inert with no inherent toxicity. However, exploring the gene expression patterns of accumulated AuNPs in liver and spleen of rat models resulted in 79 and 62 differentially expressed genes respectively, and 10 genes in common between the two organs. Classification of these

genes included several pathways including detoxification, lipid metabolism, cell cycle, defence response and circadian rhythm. This data demonstrates that the AuNPs are not entirely biologically inert and is an important consideration for the application of these materials. AuNPs can be used in further investigation of the cellular effects of Au or as a reference for the effects of other NPs in the liver and spleen.

Griffitt et al. [55] unveiled the presence of distinct biological responses from zebrafish gills when exposed to different nanoparticulate metal ions. The hypothesis was that, as these metals are toxic to the organism, a commonality existed between the response of zebrafish gills to different metal nanoparticles and/or between them and their soluble forms. This was rejected, as the data revealed by the use of microarrays found no consistent toxicological profile. The finding meant that there is no shared physiological mechanism to deal with the toxicity of metal nanoparticles and the authors warn of the implications when using toxicity data to infer the effect on different nanomaterials. In this case, microarrays were useful to rapidly determine a lack of consistency between the toxicity of different nanoparticles, useful as a negative result when studying metallic materials. It should be noted, however, that this deficiency in a toxicological profile might be limited to the specific particles and organism under study.

DNA microarray technology was used to assess both the cytotoxicity and global gene interaction between 100  $\mu\text{m}$  and 200  $\mu\text{m}$  nickel ions ( $\text{Ni}^{2+}$ ) and mouse fibroblast cells (L929) over 4 time points (12 h, 24 h, 48 h & 72 h) [56] and [57]. Nickel–titanium (NiTi) is widely used in the medical field including in orthodontics [58], root canals [59] and colon surgery [60]. However, NiTi releases nickel ions, which can cause allergic [61] and *in vitro* toxic [62] responses. The application of a gene expression profiling-based investigation enables genomic-level information above and beyond routine cytotoxicity tests. While toxicity on a cellular level was *not* detected using a methylthiazoltetrazolium (MTT) assay during the shorter time periods (12 h & 24 h), the microarray study revealed a significant change at the molecular level. This showcases the sensitivity of the microarray technology and also revealed several Gene Ontologies (GO) and pathways changed when exposed to  $\text{Ni}^{2+}$  including focal adhesion, cell cycle, insulin signalling, electron transport chain, mRNA processing and ribosome protein.

With the aim of improving the performance of iron stents, a cell culture model was designed to investigate the interaction of vascular cells and ferrous ions (Fe(II)), an iron degradation product [63]. Phenotypic and molecular examination of the effect of ferrous ions in umbilical venous smooth muscle cells (SMCs) revealed a reduced growth rate and quantity of mRNA involved in cellular proliferation with a high expression of p53 in the presence of Fe(II). Cellular membrane components were also upregulated including lipids and cholesterol. Conclusions from this study incorporated the hypothesis that ions released from iron stents could reduce the proliferation rate of vascular cells, implicating an advantageous role in restenosis *in vivo* by reducing excessive vascular cell proliferation. Combining this discovery with prospective studies on iron stents will give the investigator greater power to tailor their experiments based on the requirement for cellular proliferation.

#### *2.2.1.2. Use of microarrays in regenerative medicine*

Diverging from cell–biomaterial interactions, there is a continuously expanding field of tissue engineering, cell and gene therapies. Instead of the biomaterial passively (without integration) performing some replacement function, such as with stents, valves and pacemakers, the biomaterial is in an active state for tissue regeneration or specific stem cell or gene therapies. Biomaterial-based scaffolds have been investigated in terms of cell growth and differentiation in order to repair or replace damaged tissues. As the attempts to introduce biological activity into biomaterials increases, so does the need for comprehensive assessment of the molecular changes. Microarrays have been used to investigate the cytotoxicity of grafts as well as the potential of novel scaffolds and genes involved in the process of differentiation.

Gundy et al. [64] set out to assess the use of fibrin – a protein involved in blood clotting with the potential as a cell carrier, in combination with polylactic acid (PLA) warp-knit textile as a structural support, in order to design a synthetic graft for use in tissue engineering for coronary artery disease (CAD). Unfortunately, fibrin alone exhibits poor mechanical properties. Gene expression profiles were collected for human coronary artery smooth muscle cells (HCASMC) seeded within a fibrin gel with and without PLA. In this case, microarray results revealed that the addition of the PLA textile to fibrin did not have a significant effect on gene expression of

HCASMCs. This application of microarrays, thus, can quickly highlight the potential, or lack thereof, of novel scaffolds for tissue engineering.

Oligonucleotide arrays were used in cartilage tissue engineering to explore the differences between de- and re-differentiation between native cartilage, *in vitro* expanded chondrocytes and three-dimensionally assembled chondrocyte cultures [65]. Nine hundred and five genes were reproducibly regulated (>two-fold) indicating elemental changes that take place during cartilage tissue engineering. Results include the dedifferentiation of cartilage cells in a monolayer and re-differentiation of those chondrocytic cells in a three-dimensional assembly, verified by the expression of genes associated with metabolism and growth.

Tissue engineering is now moving into the organ regeneration arena where autologous, engineered bladder constructs were successfully used for reconstruction and implanted in patients who need cystoplasty [66]. Microarray technology has been harnessed since this to discover differences between healthy bladder smooth muscle cells (SMCs) and exstrophic SMC [67], healthy SMCs and cells from myelomeningocele [68] to characterise tissue-specific genetic signatures [69].

While microarray technology can provide an extensive mRNA profile representing the current processes taking place within a cell, follow-up with more sensitive and targeted approaches may be required to progress any potential findings. As transcripts are not translated into protein with any certainty, reflected by the lack of synchronised transcriptomic and proteomic data, it may be useful to follow up an interesting finding with a proteomic study.

#### 2.2.2. The application of proteomic technology to biomaterials

As proteins represent the current physiological status as opposed to RNA, they are more likely to reflect a more accurate molecular profile of cell–biomaterial interaction than mRNA expression alone. The investigation of a protein or protein family is no longer adequate when assessing the complexity of cell–biomaterial interactions. Proteomics has introduced the possibility of profiling a complex sample for global protein expression profile, including post-translational modifications and subcellular localisation. Mass spectrometry (MS) is an analytical technique, which is used for the

large-scale identification and quantification of proteins. The use of high-throughput proteomics techniques in the biomaterials field is still growing; indeed, only a limited number of studies using MS have been published to date.

Comparative proteomics enables the study of biocompatibility between established and altered biomaterials or the conformational changes that occur between proteins adsorbed to a biomaterial surface. Xu et al. [70] used iTRAQ-coupled 2D LC-MS/MS to profile human osteoblasts cultured on hydroxyapatite (HA). HA is biocompatible with osteoblasts but degrades over time *in vivo*. Carbon nanotube (CNT) was introduced to reinforce HA; however, the MS results were consistent with a change in its biocompatibility, displaying differentially expressed proteins involved in cell adhesion and proliferation.

Dinnes et al. [71] combined 2-dimensional electrophoresis (2-DE) with matrix-assisted laser desorption ionisation-time of flight (MALDI-ToF) to search for protein expression changes in monocyte-derived macrophages (MDM) cultured on different material surfaces. MDMs are key inflammatory cells important in the host response to a biomaterial. The protein expression changes of different MDM-biomaterial interactions were assessed with the aim to discover the cellular mechanisms behind this inflammatory response. Proteins involved in both structural remodelling and protein folding were identified as having altered expression between control and polycarbonate-urethane surfaces. Further quantitative results could progress the search for mechanisms of MDM response to different biomaterial surfaces. An increased likelihood for biomaterial success is linked to the similarity between a biomaterial and the innate system and so it is of huge benefit to be able to equate these systems.

Liquid chromatography MALDI (LC-MALDI) has also been applied to gain insight into the mechanism of monocyte-material interactions [72]. Tissue culture polystyrene (TCPS) was used as a model system to derive proteins relevant to monocyte-material responses. The Src family kinase haematopoietic cell kinase (Hck) and plasminogen activator inhibitor type 2 (PAI-2) were chosen. Then, polyethylene glycol (PEG)-only hydrogels and a PEG-based, in-house designed arginine-glycine-aspartic acid (RGD) modified semi-interpenetrating polymer network were used to investigate the surface-dependency of these proteins. There

were significant changes in expression of these and other proteins from monocytes adherent to PEG-based materials compared to TCPS.

Similar to transcriptomics, proteomic technologies have also been applied to the area of tissue engineering, where the aim is to identify factors expressed during cellular regeneration and repair [73]. Li et al. [74] used surface-enhanced laser desorption ionisation-time of flight mass spectrometry (SELDI-ToF MS), a proteinchip technology in which a protein mixture is spotted on a chip surface modified with different biological or chemical affinities. The end result is a mass-to-charge ratio of detected ions that can be compared between samples in order to look for differential expression and in this case, identified computationally using the measured molecular mass. In this study, 5 candidate proteins were identified in which the response of ear-punched tissue of a regeneration strain, MRL/MpJ-Faslpr (MRL) mice, were compared against a non-regeneration strain, C57BL/6J(B6) mice. These proteins can be further investigated as markers of repair or regeneration of soft tissue.

It is now becoming common to utilise high-throughput approaches to place results in the context of systems, pathways and ontologies to gain a more insightful view of the phenotype under study. This systems biology approach to cell–host–biomaterial interaction can help when uncovering the wide patient variability that might affect a response. A person’s current health status, lifestyle factors, age and gender only cover a portion of a large variety of potential differences between patients.

### **3. Future studies**

Future studies may aim to use imaging technology as a non-invasive method to monitor molecular changes *in vivo*. Whole-body real-time imaging has been used to evaluate host–biomaterial interactions in nuclear factor- $\kappa$ B (NF- $\kappa$ B) transgenic mice [82]. This study highlights the use of bioluminescence imaging to monitor molecules under investigation, in this case an inducible transcription factor involved in innate and adaptive immune responses. As inflammation is a vital response to monitor in host–biomaterial studies, it is important to avail of new technologies that will provide a non-invasive and whole-body status of molecular and cellular changes.

In a similar and more recent study, the same authors extended the experiments in transgenic mice by applying transcriptomic analysis to the NF- $\kappa$ B affected organs as identified by the NF- $\kappa$ B activity-driven bioluminescence [83]. This '*imaging-guided transcriptomic platform*' provides a method to identify complex host responses to organs affected by inflammation. This combination of transcriptomics and imaging technologies provides a complementary approach to cell- and host-biomaterial interaction studies.

Biomaterials in combination with high-throughput technologies also have found their place in mediating localised and controlled drug and protein delivery via nanomaterials [79] and [80]. Here, DNA-containing nanoparticles can be administered and tagged with tissue-specific genes in order to deliver gene therapy to a target of interest. As an example, Chow et al. [79] used a block copolymer with anti-inflammatory molecules as a 'copolymer-therapeutic hybrid' for a drug delivery system. Zhu et al. [81] used MS to monitor the cellular uptake of gold nanoparticles. They found that subtle changes to the surface of the gold nanoparticles could lead to significant changes in the cellular uptake, which is an important aspect when optimising drug delivery molecules.

Next-generation sequencing (NGS) is also an anticipated method for transcriptomic profiling of cell-host-biomaterial interaction and is currently appearing as a robust approach to alternative splicing and single nucleotide variation detection in conjunction with differential expression. As standard approaches to differential expression lack the sensitivity to carry out these tasks, NGS will be a more attractive approach for these purposes. NGS also offers several orders of magnitude greater dynamic range, and therefore, may have a future with biomaterial integration experiments. Fox et al. write that the application of or high-throughput sequencing is '*essentially limited only by the imaginations of researchers*' [84].

Finally, the advent of "smart" biomaterials that actively participate in the formation of functional tissue have, and will continue to play a major role in the future of tissue engineering [75]. Recently, Gerty et al. [76] have used "smart" hydrogels that are made thermoresponsive with an amphiphilic peptide-polymer conjugate which appears to provide a prolonged supportive matrix for genetically engineered stem cells. Other stimuli-responsive interfaces such as a light, temperature and pH [77] will

provide the ability for more controlled cell–biomaterial interaction (for an extensive review see [78]). To date, while high-throughput technologies have not been applied in the assessment of “smart” biomaterial–cell interaction, we expect this to be a future application.

#### **4. Bioinformatics efforts in the biomaterials arena**

The application of computerised tools in high-throughput research is essential. Throughout the majority of the articles presented in this article are programs and software packages to place gene and protein lists into biologically relevant, functional terms. Pathway analysis, gene ontologies, clustering and classifying genes helps to organise and focus experiments, especially when the study involves a global approach to biomaterial interactions. The progressing field of biomaterials in general, coupled with the advent of next-generation technologies, means the potential for an influx of data. The Biomolecular Adsorption Database (BAD) was published in 2009, maximising the protein adsorption data already produced by archiving in a central location (<http://dbweb.liv.ac.uk/bad/>) [85]. BAD currently comprises of 767 records of protein adsorption experiments reported in the literature and, in combination with neural networks and regression analysis, this information can predict the amount of adsorbed protein, the thickness of the adsorbed protein layer and the surface tension of the protein-covered surfaces. Using this initiative in other areas of biomaterials research, for example cell–biomaterial interaction, would maximise the use of expensive high-throughput technologies and the published data, reduce the complexity of multiple datasets and discover gaps in the current research.

#### **5. Conclusion**

In recent years, the biomaterial arena has become a rapidly developing field. While some progress has been made, high-throughput systems have not yet been fully harnessed in the field of biomaterials and there remains vast potential in their exploitation. High-throughput technologies have enabled researchers to move past macroscopic evaluation of their experiments towards an in-depth assessment of biocompatibility in a material. It is now possible to delve into the core of biocompatibility issues by deciphering the reactions of cells and molecules. Material

surface modifications, chemistry and roughness, hydrophobicity and physio-chemical properties all combine to synthesise unique biomaterials, which require extensive quality control checks. Moreover, the identification of subtle effects of biomaterials on cell morphology, viability, proliferation and differentiation is achievable, leading the way for future *in vivo* work. Technologies are evolving rapidly providing vital information on biomaterial use in the areas of tissue engineering, biosensor and diagnostics and targeted therapeutics. The methods described here help to determine their efficacy and a combination of HTS with gene/protein expression studies can maximise the information gain. In the era of continuously ageing population with a requirement for improved biomaterials, these studies are vital.

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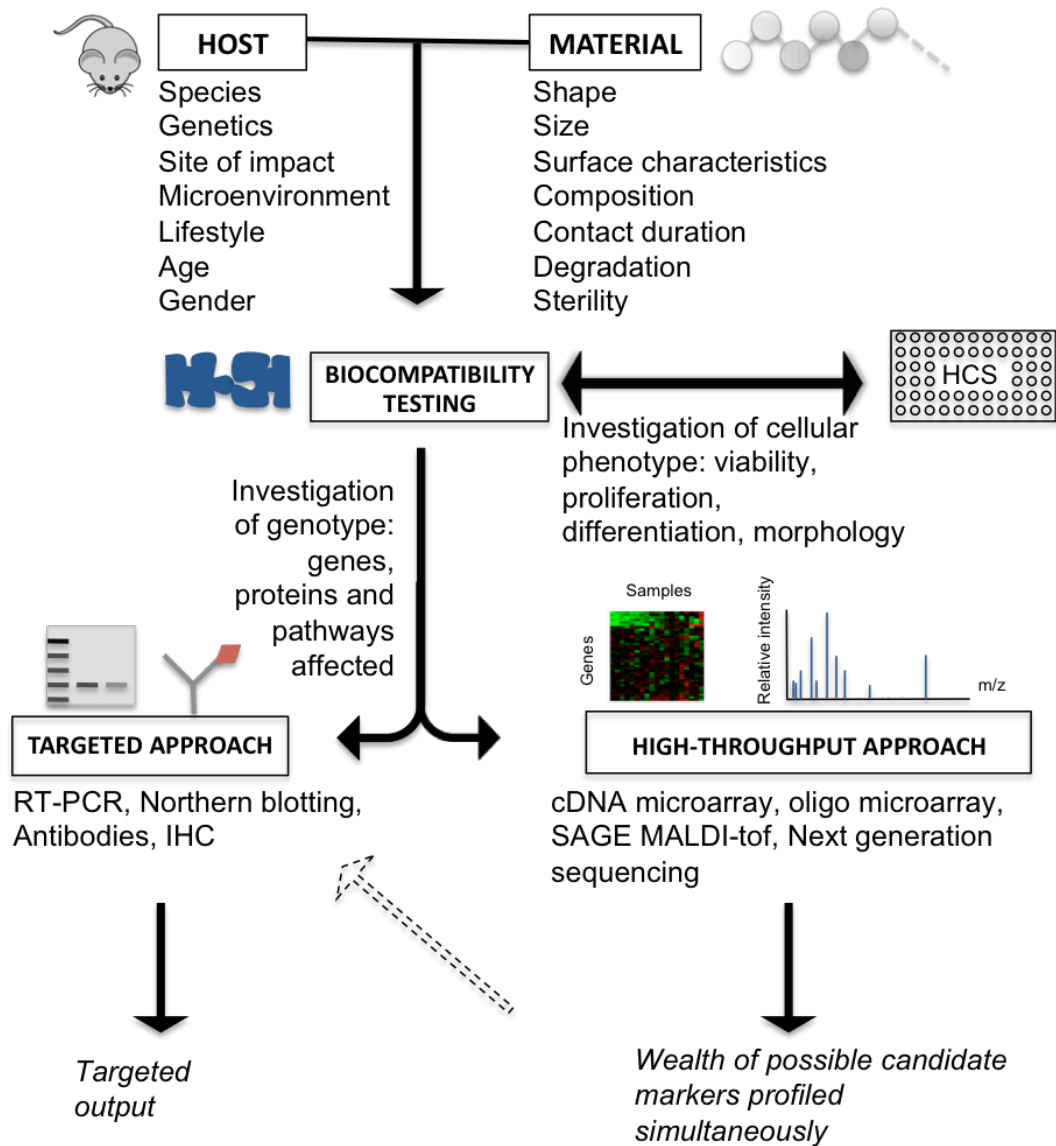
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**Figure 1. Parameters that influence the biocompatibility of an implanted material and the subsequent downstream avenues employed to investigate the host-biomaterial responses.**

**Table 1. High-throughput technologies used to profile cell-host biomaterial interaction**

<b>Technology</b>	<b>Aim</b>	<b>Result</b>	<b>Reference</b>
Cell-based screening	Identification of the most efficient polymers for transfection	Identified the most appropriate polymers for transfections (46 out of 2,350)	Anderson <i>et al.</i> 2003[45]
Cell-based screening	Screen cell lines treated with biomaterials for toxic effects	Copolymer compositions of 50% CPH induced elevated levels of TNF $\alpha$	Alder <i>et al.</i> 2009 [46]
cDNA microarray	Examine the effect of nanoparticle composition on responses of zebrafish gills after exposure to toxic nanometals	Different responses were detected among different particles	Griffittet <i>et al.</i> 2009 [55]
cDNA microarray	Investigate the molecular changes in L929 cells treated with nickel ions	118 differentially expressed genes relating to 6 main biological pathways	Lü <i>et al.</i> 2009 [56]
cDNA microarray	Investigate the cytotoxic effects of nickel ions on L929 cells	20 upregulated and 19 downregulated genes differentially expressed across 3 timepoints relating to a range of functional gene groups	Lü <i>et al.</i> 2009 [57]
cDNA microarray	Examine genes involved in the interaction between fibrin and polylactic acid	The addition of PLA to fibrin did not have significant cytotoxic effects	Grundy <i>et al.</i> 2008 [64]
Oligo microarray	Examine the biodistribution and gene expression of Au in > 25 organs of rats	AuNPs are rapidly accumulated in the liver and spleen with 79 and 62 differentially expressed genes respectively, and 10 genes in common between the two organs	Balashubramanian <i>et al.</i> 2010[54]
Oligo microarray	Investigate the interaction of vascular cells and ferrous ions	Smooth muscle cell proliferation can be controlled by ferrous ions	Mueller <i>et al.</i> 2006 [63]
Oligo microarray	Explore the differences between de- and re-differentiation between native cartilage, in vitro expanded chondrocytes and three-dimensionally assembled chondrocyte cultures	905 genes were reproducibly regulated indicating elemental changes that take place during cartilage tissue engineering.	Kapset <i>et al.</i> 2006 [65]
iTRAQ-coupled 2D LC - MS/MS	Compare the proteomic profile of human osteoblast cells cultured on plane HA and CNT reinforced HA	Proteins previously associated with cell adhesion and proliferation were found to be differentially expressed	Xuet <i>et al.</i> 2008 [70]
MALDI-ToF MS	Search for protein expression changes in MDMs cultured on different material surfaces	Proteins involved in structural remodeling and protein folding were identified as differentially expressed	Dinneset <i>et al.</i> 2006 [71]
MALDI-ToF/ToF	Investigate mechanisms underlying surface dependent expression of proteins from monocytes adherent to PEG-based material in comparison to TCPS	645 proteins detected from TCPS. Significant surface-dependent protein expression from monocytes for both PEG-based material and TCPS	Zuckerman <i>et al.</i> 2009 [72]

CPH, 1,6-bis(p-carboxyphenoxy)hexane; HA, hydroxyapatite; CNT, carbon nanotube; MDM, Monocyte-derived macrophages; L929, mouse fibroblast cell line; ITRAQ, sobaric tag for relative and absolute quantitation; MALDI, matrix-assisted laser desorption/ionization; ToF, time-of-flight; MS, mass spectrometry; PEG, poly(ethylene glycol); TCPS, tissue culture poly(styrene).