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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 210 times in PubMed [11] which is 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-β1) 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 results 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, l929 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 µm and 200 µm nickel ions (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 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-Faslrpr (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-κB (NF-κB) 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-κB affected organs as identified by the NF-κB 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 thermoreponsive 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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References

1. Neidle S, Thurston DE. Chemical approaches to the discovery and development of cancer therapies. Nat Rev Cancer. 2005;5[4]:285-296.

2. Ocak S, Sos ML, Thomas RK, Massion PP. High-throughput molecular analysis in lung cancer: insights into biology and potential clinical applications. Eur Respir J. 2009;34[2]:489-506.

3. Rowan K. High-Throughput Screening Finds Potential Killer of Cancer Stem Cells. J. Natl. Cancer Inst. 2009;101[21]:1438-1439.

4. Sims AH. Bioinformatics and breast cancer: what can high-throughput genomic approaches actually tell us? Journal of Clinical Pathology. 2009;62[10]:879-885.

5. Kash JC. Applications of high-throughput genomics to antiviral research: evasion of antiviral responses and activation of inflammation during fulminant RNA virus infection. Antiviral Res. 2009;83[1]:10-20.

6. Peters A, Brey DM, Burdick JA. High-throughput and combinatorial technologies for tissue engineering applications. Tissue Eng Part B Rev. 2009;15[3]:225-239.

7. Nishant KT, Singh ND, Alani E. Genomic mutation rates: what high-throughput methods can tell us. Bioessays. 2009;31[9]:912-920.

8. Etzion Y, Muslin AJ. The application of phenotypic high-throughput screening techniques to cardiovascular research. Trends Cardiovasc. Med. 2009;19[6]:207-212.

9. The ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007;447:799-816.

10. Gallagher WM, Lynch I, Allen LT, Miller I, Penney SC, O'Connor DP, et al. Molecular basis of cell-biomaterial interaction: insights gained from transcriptomic and proteomic studies. Biomaterials. 2006;27[35]:5871-5882.
11. Vastag B. NIH Launches PubMed Central. J. Natl. Cancer Inst. 2000;92[5]:374.

12. Lee K, Goodman SB. Current state and future of joint replacements in the hip and knee. Expert Rev Med Devices. 2008;5[3]:383-393.

13. Kashuk KB, Haber E. Tendon and ligament prostheses. Clin Podiatry. 1984;1[1]:131-143.

14. Mol A, Smits AIPM, Bouten CVC, Baaijens FPT. Tissue engineering of heart valves: advances and current challenges. Expert Rev Med Devices. 2009;6[3]:259-275.

15. Esposito M, Murray-Curtis L, Grusovin M, Coulthard P, Worthington H. Interventions for replacing missing teeth: different types of dental implants. In: Cochrane Database of Systematic Reviews. 2007.

16. Stapleton F, Stretton S, Papas E, Skotnitsky C, Sweeney DF. Silicone hydrogel contact lenses and the ocular surface. Ocul Surf. 2006;4[1]:24-43.

17. Scott M. 32,000 years of sutures. NATNEWS. 1983;20[5]:15-17.

18. Jones KS. Effects of biomaterial-induced inflammation on fibrosis and rejection. Semin. Immunol. 2008;20[2]:130-136.

19. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. The role of complement in biomaterial-induced inflammation. Mol. Immunol. 2007;44[1-3]:82-94.

20. Guo K, Chu C. Biodegradation of unsaturated poly(ester-amide)s and their hydrogels. Biomaterials. 2007;28[22]:3284-3294.

21. Roach P, Farrar D, Perry CC. Interpretation of protein adsorption: surface-induced conformational changes. J. Am. Chem. Soc. 2005;127[22]:8168-8173.
22. Shen J, Wu T, Wang Q, Pan H. Molecular simulation of protein adsorption and desorption on hydroxyapatite surfaces. Biomaterials. 2008;29[5]:513-532.

23. Courtney JM, Zhao XB, Qian H, Sharma A. Modification of polymer surfaces: optimization of approaches. Perfusion. 2003;18 Suppl 1:33-39.

24. Allen LT, Tosetto M, Miller IS, O'Connor DP, Penney SC, Lynch I, et al. Surface-induced changes in protein adsorption and implications for cellular phenotypic responses to surface interaction. Biomaterials. 2006;27[16]:3096-3108.

25. Keselowsky BG, Collard DM, García AJ. Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion. J Biomed Mater Res A. 2003;66[2]:247-259.

26. Meyers SR, Khoo X, Huang X, Walsh EB, Grinstaff MW, Kenan DJ. The development of peptide-based interfacial biomaterials for generating biological functionality on the surface of bioinert materials. Biomaterials. 2009;30[3]:277-286.

27. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008;29[20]:2941-2953.

28. Park H, Cannizzaro C, Vunjak-Novakovic G, Langer R, Vacanti CA, Farokhzad OC. Nanofabrication and microfabrication of functional materials for tissue engineering. Tissue Eng. 2007;13[8]:1867-1877.

29. Langer R, Vacanti JP. Tissue engineering. Science. 1993;260[5110]:920-926.

30. Ravi S, Chaikof EL. Biomaterials for vascular tissue engineering. Regen Med. 2010;5[1]:107-120.

31. Le SJ, Gongora M, Zhang B, Grimmond S, Campbell GR, Campbell JH, et al. Gene expression profile of the fibrotic response in the peritoneal cavity. Differentiation. 2010;10.1016/j.diff.2010.03.001.
32. Ulici V, James CG, Hoenselaar KD, Beier F. Regulation of Gene Expression by PI3K in Mouse Growth Plate Chondrocytes. PLoS ONE. 2010;5[1]:e8866.

33. Minogue BM, Richardson SM, Zeef LA, Freemont AJ, Hoyland JA. Transcriptional profiling of bovine intervertebral disc cells: implications for identification of normal and degenerate human intervertebral disc cell phenotypes. Arthritis Res Ther. 2010;12[1]:R22.

34. Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev. Mol. Diagn. 2008;8[6]:707-725.

35. Kallioniemi OP, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high-throughput molecular profiling of cancer. Hum. Mol. Genet. 2001;10[7]:657-662.

36. Vince D, Hunt JA, Williams DF. Quantitative assessment of the tissue response to implanted biomaterials. Biomaterials. 1991;12[8]:731-736.

37. Hunt JA, McLaughlin PJ, Flanagan BF. Techniques to investigate cellular and molecular interactions in the host response to implanted biomaterials. Biomaterials. 1997;18[22]:1449-1459.

38. Zhang H, Aronow MS, Gronowicz GA. Transforming growth factor-beta 1 (TGF-beta1) prevents the age-dependent decrease in bone formation in human osteoblast/implant cultures. J Biomed Mater Res A. 2005;75[1]:98-105.

39. Hammermeister KE, Sethi GK, Henderson WG, Oprian C, Kim T, Rahimtoola S, et al. A Comparison of Outcomes in Men 11 Years after Heart-Valve Replacement with a Mechanical Valve or Bioprosthesis. N Engl J Med. 1993;328[18]:1289-1296.

40. Flanagan TC, Pandit A. Living artificial heart valve alternatives: a review. Eur Cell Mater. 2003;6:28-45; discussion 45.

41. Ramirez CN[, Antczak C[, Djaballah H[. Cell viability assessment: toward content-rich platforms. Expert Opinion on Drug Discovery. 2010;5:223-233.
42. Blackmore MG, Moore DL, Smith RP, Goldberg JL, Bixby JL, Lemmon VP. High content screening of cortical neurons identifies novel regulators of axon growth. Mol. Cell. Neurosci. 2010;44[1]:43-54.

43. Daub A, Sharma P, Finkbeiner S. High-content screening of primary neurons: ready for prime time. Curr. Opin. Neurobiol. 2009;19[5]:537-543.

44. Yliperttula M, Chung BG, Navaladi A, Manbachi A, Urtti A. High-throughput screening of cell responses to biomaterials. Eur J Pharm Sci. 2008;35[3]:151-160.

45. Anderson DG, Lynn DM, Langer R. Semi-Automated Synthesis and Screening of a Large Library of Degradable Cationic Polymers for Gene Delivery. Angewandte Chemie International Edition. 2003;42[27]:3153-3158.

46. Adler AF, Petersen LK, Wilson JH, Torres MP, Thorstenson JB, Gardner SW, et al. High throughput cell-based screening of biodegradable polyanhydride libraries. Comb. Chem. High Throughput Screen. 2009;12[7]:634-645.

47. Yang F, Mei Y, Langer R, Anderson DG. High throughput optimization of stem cell microenvironments. Comb. Chem. High Throughput Screen. 2009;12[6]:554-561.

48. Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. J. Biomed. Mater. Res. 2001;55[2]:151-157.

49. Carinci F, Pezzetti F, Volinia S, Francioso F, Arcelli D, Farina E, et al. Zirconium oxide: analysis of MG63 osteoblast-like cell response by means of a microarray technology. Biomaterials. 2004;25[2]:215-228.

50. Dalby MJ, Yarwood SJ, Riehle MO, Johnstone HJH, Affrossman S, Curtis ASG. Increasing fibroblast response to materials using nanotopography: morphological and genetic measurements of cell response to 13-nm-high polymer demixed islands. Exp. Cell Res. 2002;276[1]:1-9.
51. Ku C, Browne M, Gregson PJ, Corbeil J, Pioletti DP. Large-scale gene expression analysis of osteoblasts cultured on three different Ti-6Al-4V surface treatments. Biomaterials. 2002;23[21]:4193-4202.

52. Kato S, Kishida A, Hanyu N, Maruyama I, Akashi M. Study of cellular responses to polymeric biomaterials using the differential display method. J Biomater Sci Polym Ed. 2000;11[4]:333-340.

53. Garrigues GE, Cho DR, Rubash HE, Goldring SR, Herndon JH, Shanbhag AS. Gene expression clustering using self-organizing maps: analysis of the macrophage response to particulate biomaterials. Biomaterials. 2005;26[16]:2933-2945.

54. Balasubramanian SK, Jittiwat J, Manikandan J, Ong C, Yu LE, Ong W. Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials. 2010;31[8]:2034-2042.

55. Griffitt RJ, Hyndman K, Denslow ND, Barber DS. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. Toxicol. Sci. 2009;107[2]:404-415.

56. Lü X, Lu H, Zhao L, Yang Y, Lu Z. Genome-wide pathways analysis of nickel ion-induced differential genes expression in fibroblasts. Biomaterials. 2010;31[8]:1965-1973.

57. Lü X, Bao X, Huang Y, Qu Y, Lu H, Lu Z. Mechanisms of cytotoxicity of nickel ions based on gene expression profiles. Biomaterials. 2009;30[2]:141-148.

58. Burstone CJ, Qin B, Morton JY. Chinese NiTi wire--a new orthodontic alloy. Am J Orthod. 1985;87[6]:445-452.

59. Torrisi L. The NiTi superelastic alloy application to the dentistry field. Biomed Mater Eng. 1999;9[1]:39-47.

60. Domingo S, Puértolas S, Gracia-Villa L, Mainar M, Usón J, Puértolas JA. Design, manufacture and evaluation of a NiTi stent for colon obstruction. Biomed Mater
61. Hosoki M, Bando E, Asaoka K, Takeuchi H, Nishigawa K. Assessment of allergic hypersensitivity to dental materials. Biomed Mater Eng. 2009;19[1]:53-61.

62. Kao C, Ding S, He H, Chou MY, Huang T. Cytotoxicity of orthodontic wire corroded in fluoride solution in vitro. Angle Orthod. 2007;77[2]:349-354.

63. Mueller PP, May T, Perz A, Hauser H, Peuster M. Control of smooth muscle cell proliferation by ferrous iron. Biomaterials. 2006;27[10]:2193-2200.

64. Gundy S, Manning G, O'Connell E, Ellä V, Harwoko MS, Rochev Y, et al. Human coronary artery smooth muscle cell response to a novel PLA textile/fibrin gel composite scaffold. Acta Biomater. 2008;4[6]:1734-1744.

65. Kaps C, Frauenschuh S, Endres M, Ringe J, Haisch A, Lauber J, et al. Gene expression profiling of human articular cartilage grafts generated by tissue engineering. Biomaterials. 2006;27[19]:3617-3630.

66. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet. 2006;367[9518]:1241-1246.

67. Hipp J, Andersson K, Kwon TG, Kwak EK, Yoo J, Atala A. Microarray analysis of extrophic human bladder smooth muscle. BJU Int. 2008;101[1]:100-105.

68. Hipp JA, Hipp JD, Yoo JJ, Atala A, Andersson K. Microarray analysis of bladder smooth muscle from patients with myelomeningocele. BJU Int. 2008;102[6]:741-746.

69. Lu S, Hipp JA, Feng Q, Hipp JD, Lanza R, Atala A. GeneChip analysis of human embryonic stem cell differentiation into hemangioblasts: an in silico dissection of mixed phenotypes. Genome Biol. 2007;8[11]:R240.

70. Xu J, Khor KA, Sui J, Zhang J, Tan TL, Chen WN. Comparative proteomics profile of osteoblasts cultured on dissimilar hydroxyapatite biomaterials: an iTRAQ-coupled 2-D LC-MS/MS analysis. Proteomics. 2008;8[20]:4249-4258.
71. Dinnes DLM, Marçal H, Mahler SM, Santerre JP, Labow RS. Material surfaces affect the protein expression patterns of human macrophages: A proteomics approach. J Biomed Mater Res A. 2007;80[4]:895-908.

72. Zuckerman ST, Brown JF, Kao WJ. Identification of regulatory Hck and PAI-2 proteins in the monocyte response to PEG-containing matrices. Biomaterials. 2009;30[23-24]:3825-3833.

73. Metcalfe AD, Ferguson MWJ. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J R Soc Interface. 2007;4[14]:413-437.

74. Li X, Mohan S, Gu W, Miyakoshi N, Baylink DJ. Differential protein profile in the ear-punched tissue of regeneration and non-regeneration strains of mice: a novel approach to explore the candidate genes for soft-tissue regeneration. Biochim. Biophys. Acta. 2000;1524[2-3]:102-109.

75. Furth ME, Atala A, Van Dyke ME. Smart biomaterials design for tissue engineering and regenerative medicine. Biomaterials. 2007;28[34]:5068-5073.

76. Garty S, Kimelman-Bleich N, Hayouka Z, Cohn D, Friedler A, Pelled G, et al. Peptide-Modified "Smart" Hydrogels and Genetically Engineered Stem Cells for Skeletal Tissue Engineering. Biomacromolecules [Internet]. 2010 12 [cited 2010 14];Available from: http://www.ncbi.nlm.nih.gov/pubmed/20462241

77. Truong MY, Dutta NK, Choudhury NR, Kim M, Elvin CM, Hill AJ, et al. A pH-responsive interface derived from resilin-mimetic protein Rec1-resilin. Biomaterials. 2010;31[15]:4434-4446.

78. Cole MA, Voelcker NH, Thissen H, Grierser HJ. Stimuli-responsive interfaces and systems for the control of protein-surface and cell-surface interactions. Biomaterials. 2009;30[9]:1827-1850.

79. Chow EK, Piestorff E, Cheng G, Ho D. Copolymeric Nanofilm Platform for Controlled and Localized Therapeutic Delivery. ACS Nano. 2008;2[1]:33-40.
80. Agarwal A, Mallapragada SK. Synthetic sustained gene delivery systems. Curr Top Med Chem. 2008;8[4]:311-310.

81. Zhu Z, Ghosh PS, Miranda OR, Vachet RW, Rotello VM. Multiplexed screening of cellular uptake of gold nanoparticles using laser desorption/ionization mass spectrometry. J. Am. Chem. Soc. 2008;130[43]:14139-14143.

82. Ho T, Chen Y, Hsiang C. Noninvasive nuclear factor-kappaB bioluminescence imaging for the assessment of host-biomaterial interaction in transgenic mice. Biomaterials. 2007;28[30]:4370-4377.

83. Hsiang C, Chen Y, Ho T. Nuclear factor-kappaB bioluminescence imaging-guided transcriptomic analysis for the assessment of host-biomaterial interaction in vivo. Biomaterials. 2009;30[17]:3042-3049.

84. Fox S, Filichkin S, Mockler TC. Applications of ultra-high-throughput sequencing. Methods Mol. Biol. 2009;553:79-108.

85. Vasina EN, Paszek E, Nicolau, Jr DV, Nicolau DV. The BAD project: data mining, database and prediction of protein adsorption on surfaces. Lab Chip. 2009;9[7]:891.
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

| Technology                       | Aim                                                                 | Result                                                                 | Reference |
|----------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------|-----------|
| Cell-based screening             | Identification of the most efficient polymers for transfection      | Identified the most appropriate polymers for transfections (46 out of 2,350) | Anderson et al. 2003[45] |
| Cell-based screening             | Screen cell lines treated with biomaterials for toxic effects        | Copolymer compositions of 50% CPH induced elevated levels of TNFα       | Alder et al. 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             | Griffith et al. 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 et al. 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 et al. 2009 [57] |
| Oligo microarray                 | Examine genes involved in the interaction between fibrin and polyactic acid | The addition of PLA to fibrin did not have significant cytotoxic effects | Grundy et al. 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 | Balasubramanian et al. 2010[54] |
| Oligo microarray                 | Investigate the interaction of vascular cells and ferrous ions       | Smooth muscle cell proliferation can be controlled by ferrous ions      | Mueller et al. 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. | Kaps et al. 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 al. 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 al. 2006 [71] |
| MALDI-ToF/ToF                    | Investigate 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 et al. 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).