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REVIEW

Device Processing Challenges for Miniaturized Sensing Systems Targeting Biological Fluids

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Abstract

This article presents a review of device processing technologies used in the fabrication of biomedical systems, and highlights the requirements of advanced manufacturing technology. We focus on biomedical systems that perform diagnostics of fluidic specimens, with analytes that are in the liquid phase. In the introduction, we define biomedical systems as well as their versatile applications and the essential current trends. The paper gives an overview of the most important biomolecules that typically must be detected or analyzed in several applications. The paper is structured as follows. First, the conventional architecture and construction of a biosensing system is introduced. We provide an overview of the most common biosensing methods that are currently used for the detection of biomolecules and its analysis. We present an overview of reported biochips, and explain the technology of biofunctionalization and detection principles, including their corresponding advantages and disadvantages. Next, we introduce microfluidics as a method for delivery of the specimen to the biochip sensing area. A special focus lies on material requirements and on manufacturing technology for fabricating microfluidic systems, both for niche and mass-scale production segments. We formulate requirements and constraints for integrating the biochips and microfluidic systems. The possible impacts of the conventional microassembly techniques and processing methods on the entire biomedical system and its specific parts are also described. On that basis, we explain the need for alternative microassembly technologies to enable the integration of biochips and microfluidic systems into fully functional systems.

Keywords Biomedical system · Biosensors · Biological analysis · Alternative microassembly techniques

Introduction

There is an increasing demand for systems capable of performing biological analyses [1], such as biomolecules' detection devices and versatile assays. The biological analyzes generally consist in the detection of specific biomolecules in different medium which often requires biochemical reactions [2] such as PCR, DNA hybridization, RNA sequencing etc.

Typical examples of analytes for biological analyses can be biomolecules, cells, viruses, and bacteria. By biological molecules [3] or just biomolecules, one understands any of numerous substances that are produced by, found in, or occur naturally in any living organisms and cells. The biomolecules can be classified in 4 main groups: proteins, carbohydrates, lipids and nucleic acids (DNA and RNA).

Viruses are combination of different types of molecules that consist of genetic material such as DNA and/or RNA, as well as a protein coat. The most known examples of viruses are the large family of influenza and corona viruses [4], herpes, Ebola, Zika viruses etc.

The viruses are not living organisms and need a host to survive. They can't reproduce on their own like bacteria. Bacteria are free-living cells comprising genetic information (such as RNA and DNA) which can cause infection diseases. During the analysis, viruses must be clearly distinguished from bacteria, other living cells and biomolecules [5].

Sensors that are capable of detecting biomolecules, viruses or cells are commonly called biochips or biosensors [6]. A hybrid platform or holistic system that transfers the biological signal into an electrical signal and finally to understandable information for humans is often referred to as a lab-on-chip (LoC) in the literature [7], or lab-on-PCB [8], both being part of the more general class of biomedical systems [9].

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Depending on their intended use, the diagnostic systems can be multiple- or single- use [10]. The single use system often is called a disposable. The cost of such systems is always a concern, and strongly linked to the cost model that is based on manufacturing volume. For example, in the case of the single use self-test which detects the presence of the corona virus and which can be used by anyone and is produced at a large scale, the cost overcomes the accuracy. Conversely, more accurate testing, requires expensive and multiple use sophisticated laboratory systems run by trained personnel. Between those two extreme cases, there is a wide spectrum of diagnostic systems that are relatively quick and sufficiently accurate for specific intended purposes. Environmental considerations [11] are also a great concern, especially for single use quick testing kits that are mass produced. It is mandatory that these are easily recyclable and reduce as much as possible the ensuing environmental impact: both criteria need to be considered at the early stages of the design. On the other hand, multiple use systems comprising several parts can undergo a separate recycling phases for the individual components of such system, following conventional electronic waste recycling protocols.

Currently, various types of biosensors are used in healthcare, wearable applications and clinical research, and for different related scientific purposes. The development of biomedical systems is supported by multidisciplinary approaches and emerging technologies including the most advanced techniques and processing used in micro- and nano-electronics, microsystems and microfluidics technology.

In our review, we will discuss different biosensors used for bioanalysis and various biosensing applications. Among the latter, we will define the most common architectures and designs for the biosensors, describe which materials they are made of and formulate requirements for such materials. Special attention will be paid to assembly and integration technologies used for biosensing systems fabrication for low-, medium- and mass-scale production. We will also discuss the environmental impact of the described materials and technologies.

Biosensing System

There are several biomedical systems intended for biomolecules' detection and analysis in liquid phase [10]. Despite such variety, it is possible to identify a common architecture to most of these systems. This architecture comprises three main functional parts [12], as illustrated in Fig. 1.

The first part is the biochip, which is used for the biomolecules' detection itself. Through biofunctionalization, this biochip is made sensitive to a specific biological signal and converts it using a selected transduction principle into an electrical signal. The second part is a delivery system supplying and eventually dosing the test specimen on the sensing part of the chip. The delivery system, because of the very low volume of the liquid specimen, is often called a microfluidics system. Finally, the third part of the system is the electronic readout which converts the electrical signal into human readable data. It can also include a data processing, transmission and/or storage unit.

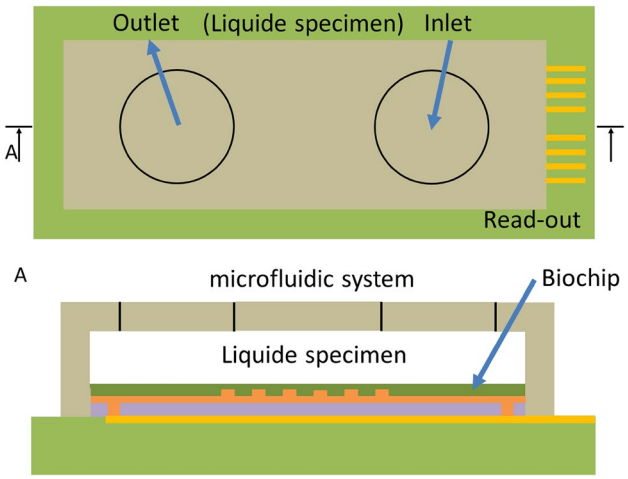

Fig. 1 Typical architecture of a biomedical system for in-liquid analysis (top and cross-sectional view)

Biochips Definition

The biochip is commonly defined as a small-scale engineered system to conduct varieties of biochemical reactions on biological samples aiming numbers of biological applications. It can be any sort of biological analysis, as a simple recognition or detection of the presence of specific biomolecules in different medium such viruses or bacteria. Typical examples of such biological analyses [10] are PCR, DNA [5] and RNA hybridization [13], DNA strain shearing etc.

A biochip depends on a mode of detection and converting a biological signal into easily human-readable and processable electrical signal can classified in different groups based on their transduction principle [5]. Among them the most common are electrical and electrochemical, magnetic and optical biochips.

Biofunctionalization

The most traditional approach to make a die to perform a biological function is to conduct a biofunctionalization [14, 15]. The biofunctionalization is a form of a given surface modification that can include chemical and physical modifications [16]. The process is based on a multidisciplinary approach, including the most advanced achievements of

micro- and nanotechnologies [17]. Biofunctionalization targets to create a biological interaction between the resulted biofunctionalized surface and a specific target biomolecule. The biofunctionalization is achieved by immobilization of varieties of biomolecules [18]. Typical biomolecules used for such purpose are proteins [19, 20], peptides [21], polyethylene glycol (PEG) [21] and others [22] such as collagen, hydrogel, gelatin and morphogenetic protein [23]. The result of the biofunctionalization is that the die obtains specificity of the die sensor surface.

The biofunctionalization of some materials such Ti and some Ti alloys for example [24], can use the natural ability of Ti [25] to form a non-stoichiometric TiO2. The oxide surface reacts with water molecules and forms hydroxyl groups that enable binding molecules. Another method uses the ability of calcium phosphate to form naturally passive groups on Ti and its alloys. Similar methods of biofunctionalization are possible on aluminum and natural aluminum oxide [26]. Another widely used technique is a silanization [27] that can enable or substantially improve adhesion a biofunctional layer such peptides and proteins on varieties of surfaces including glass [27], quartz and other material widely used for biomedical applications. The result of the silanization is an amine functionality that has a strong bond with most molecules that are used for biofunctionalization. Silanization [28] is widely used and performed by means of an aminosilane compound, specifically by 3-aminopropyl trimethoxysilane (APTES). Hence other researchers are looking for alternatives to improve adhesion between different surface and biomaterials [29]. Before the processing the surface must have already –OH, –O or related groups that are necessary to bond with the corresponding APTES group. The –OH and other groups form either naturally or as the result of an additional processing such as, for instance, plasma treatment. The silanization can be performed using a gas (for example, a gas phase silanization in a tube at 80 °C [29]) or a liquid [28], for example 1–2% APTES in deionized water [30]. The silanization process is conceptually presented in Fig. 2. The corresponding APTES groups react with –OH group that presents already on the substrates, the result of that is an ammine group $–NH_2$, then a biomolecule bonds to the amine group. Deschaume et al. [29] reports on proteins and peptides density grafted on silane layers obtained on silicon wafer.

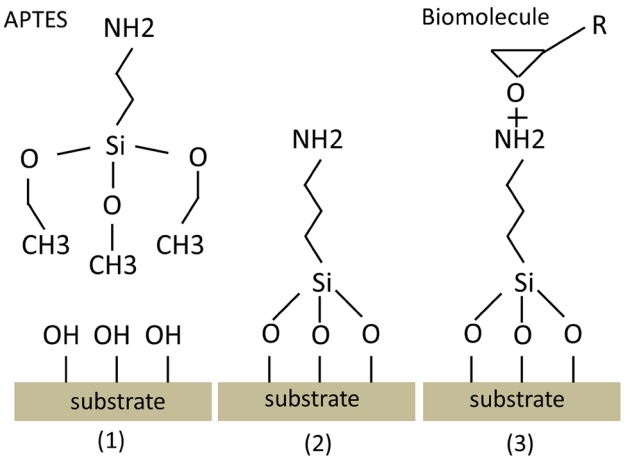

Fig. 2 Silanization with APTES: **1** APTES and substrate surface with –OH groups, **2** APTES bonds to the surface and creates amine functionality, **3** biomolecules bond to amine group

Biosensor Technology

Impedance Biosensor

Biofunctionalized impedance-based biosensors are among the most widely used sensors for the detection of bacteria [31]. The sensing part of the impedance biosensor consists of a relatively simple and well-studied structure as interdigitated electrodes (IDE) [32]. They are patterned on a metal thin film, processed on silicone die using CMOS or MEMS processing platform. IDE, or so-called a comb drive structures, are widely used for several non-biological sensing applications such a gas and air flow, moisture and humidity measurement [33]. There are at least two main groups of IDE-based sensors. In the first group, IDE are fixed and have no any degree of movement. In the second group [32], one set of the comb electrodes is fixed, whereas a complimentary set of comb electrodes has a certain freedom of movement that is defined and controlled by a spring suspension. The freedom of movement for the other set of comb electrodes extends its application area to sensing of movements, accelerations, inclination and pressure. The impedance sensor with fixed IDE can be relatively easy converted into biosensor by modification of the sensor surface by a biological layer. However, there is no application of the sensor with IDE with a freedom of movement to biosensing.

The most straightforward method is to use intrinsic properties of biomolecules and other analytes, identified as label-free methods. Yi et al. [34] performs extensive theoretical and experimental studies on capacitive structures and demonstrates that the system can detect, using a label-free method, the presence of a single stranded DNA. Kyu Kim et al. [35] demonstrate that biosensors with similar structures perform label-free detection of biomolecular interactions.

However, it is not always possible to detect biomolecules based on their properties; also to increase the sensor sensitivity, a corresponding label must be used. The labels selectively interact with their target biomolecules so these can be easily detected and quantified by the sensor. To increase sensitivity of IDE and other micro- nano-gap structures, researchers successfully explored and demonstrated several

approaches of binding corresponding labels to analyzed biomolecules. For example, in [26], gold nano-particles were used as labels bonded to DNA to increase the electrical output signal. Gold particles were also used in an immunoassay [36] towards influenza virus detection using a biofunctionalized alumina-coated capacitive biosensor. Despite the proven efficiency of the biofunctionalization, it has several disadvantages. From functionality point of view, the biofunctional layer typically has specificity to strictly particular type of biomolecules, practically meaning that it can detect only one very specific type of them. Additionally, the process of biofunctionalization is a costly method because of several factors. In principle, the biofunctionalization can be performed on the wafer-level, however after that the wafer must undergo several assembly steps that damages its integrity and as a result of that the functionality of the biofunctional layer itself. We will give more information on typical techniques and processing methods used for assembly biosensing systems, in the Section "Assembly and Integration Methods". Because of the potential damage caused by post-biofunctionalization processing, the biofunctional layer is often applied after full or partial system assembly on each die individually, so-called die-level process. Often the biofunctional layer remains active only for one cycle of assay, and after each cycle of detection, the biofunctional layer must be reapplied on the biochip surface. Therefore, there is an increased interest to develop biosensing systems which don't require the biofunctionalization.

The authors of [37, 38] performed an extensive study of various parameters of capacitive sensor to achieve maximum sensitivity to bacteria. They studied different IDE configurations, for instance electrodes with different widths and gaps, as well as the thickness of the Al electrodes and the dielectric configuration on top of the electrodes. They also showed that the permittivity and conductivity of the electrolyte used to deliver the bacteria to the sensing area might have an important effect. They found that not all bacterial cells can be quantified by this method: a minimal cytoplasm conductivity is required to obtain the required sensitivity. The bacteria diameter is also important; however a corresponding IDE configuration can address this point. This method requires no biofunctionalization and is a purely label-free method [39].

Magnetic Biosensors

Several research publications report on the use of magnetic micro- and nano-beads as a label to analyze biomolecules. Similar to conductive beads and using the same principles the magnetic beads conjugate only to specific targeted biomolecules.

The sensor sensing part has typically an integrated conductive microstructure that is quite similar to the IDE that are used in the impedance-based sensors. Unlike capacitive sensors, for which the detection principle is based on changes in the sensor impedance such as capacitance and/or conductivity, the sensor detects magnetic phenomena. The biomolecules labeled with the magnetic beads above the IDE causing a magnetic field disturbance resulting in a change in the resistance of the sensor. The choice of diameter of the magnetic beads is based on targeted molecules dimensions. The features of the integrated conductive microstructures, such as the width and gap, also depend on the analyzed molecules dimensions. The final step in the biodetection is applying the magnetic field by coil-inductance or by an external magnet as high as 1T. Because of the strong magnetic field, the biomolecules conjugated with the magnetic beads are separated from any other presented in the analyzed solution. In [40], the authors use this method to label and then separate cancer blood cells (leukemia). They used superparamagnetic polystyrene micro-particles of 4.5 μm diameter functionalized with a monoclonal mouse antibody specific for the membrane antigen, which is pre-dominantly expressed on human T cells.

In [41], the authors demonstrated the magnetoresistive-based biosensors that can be used for molecule detection and recognition. The technique is based on the detection of a magnetically labeled biomolecule interacting with a biofunctionalized magnetic-field sensor in the external strong magnetic field provided by two magnets. Another work by Gokere et al. [42] made an extra step forward and developed a label-free magnetic levitation-based assay that detects density differences of individual red blood cells down to 0.0001 g/mL resolution. The main advantages of the assay is that it does not require magnetic particles and corresponding biofunctionalization.

Optical Biosensors

Recently, Sharma et al. [43] described the main advantages of the optical sensors for biomolecules detection. Whereas conventional detection methods e.g. enzyme antibody, PCR assay or immunofluorescence microscopy, can last for several hours or even days [43], optical detection methods can deliver results much quicker [44].

As mentioned in Section "Biochip functionality", one of the important trends in the detection of biomolecules is the label-free detection technique [45]. Such technique generates a detectable signal directly upon binding to the sensing surface. The label-free technique does not need any additional interaction with an agent that carries a corresponding label. The optical methods can provide a direct label-free detection. Currently the most common method [45] for optical label-free detection is surface plasmon resonance (SPR). The plasmon wave interacts with the adsorbed molecules which causes changes in the surface plasmon waves

parameters such as the refraction index and the resonance frequency. The SPR method can be used alone or with other detecting techniques, e.g. with a SAW sensor [46]. Such combined methods can improve the device performance and gain required sensitivity.

Apart from SPR, other optical methods need to be mentioned, such as interferometry, ellipsometry measurements, thin layer chromatography; ring- and micro-resonators based biosensors [47] etc. which are using varieties of detection systems including optical components such as photonic crystals, fiber-optics and surface planar optical waveguides [43].

One of the most significant disadvantages [43] of the optical sensing methods for the detection of large biomolecules is the detection penetration depth, which is usually less than the average size of a bacterial cell. That being said, such methods are very promising for smaller size of the biomolecules.

SAW Biosensors

Another large group of biochips use SAW sensors, which are described in multiple studies [48]. SAW biosensors are suitable not only for versatile chemical sensing [49], but also for in-liquid biological and/or biochemical analysis. The method is based on the accurate detection of a disturbance within an acoustic wave, occurring when the analyte comes into contact with the surface on which the acoustic wave is propagating. The acoustic wave parameters, typically amplitude and/or phase, shift because of specific biomolecules' presence on the acoustic wave-guide. Matatagui et al. [50] demonstrated that SAW is a very fast, sensitive and label-free detection method for the grow factor detection.

Ten et al. [51] reported on the use of SAW sensors for the detection and manipulation of cells, and the quantification on proteins, vapor molecules and DNA [52] hybridizations.

Friedt and Francis [53] used a combination of SAW and optical measurements to identify a water content in collagen and protein layers. Wang et al. [54] explained in detail the theoretical aspects and the modeling principles for SAW propagation, and their use for the detection of biomolecules.

The challenges for the SAW sensors are well understood and known, among them Huang et al. [48] mentioned biofouling. Biofouling typically affects the sensitivity and the specificity of the corresponding detection.

From an instrumental point of view, because of the difference between the biomolecules such as macromolecules (such as a protein, nucleic acid, lipids, polysaccharides etc.) and micromolecules (as nucleotides, amino acids, monosaccharides, fatty acids etc.), these impact the SAW propagation differently, and the corresponding response must be calibrated accordingly. Eventually not all type of SAW sensors are suitable for detecting varieties of biomolecules.

Another issue specifically related to SAW sensors was addressed in papers [55, 56], namely how to develop reproducible fluidic systems that will cause a low acoustic leakage and disturbance for SAW propagation. Stoukatch et al. [57] described in detail a low cost manufacturing process for a microfluidic device by micromilling technology. It was sequentially demonstrated in a newly developed process for the integration of the microfluidic system with a SAW sensor and proved that the realized fluidic system causes little or no disturbance for SAW propagation.

A representative example of the SAW biomolecule detection system assembled on a PCB is depicted in Fig. 3. The system comprises a SAW quartz sensor processed using conventional thin-film technology and an integrated polycarbonate micromachined microfluidic system.

Several works report on using different materials for manufacturing fluidic systems using SAW sensors, as for example in [58].

Other Detection Methods

To finish this section, we would like to mention a few alternative methods for the detection of biomolecules. Among them, the most important are fluorescently labeled target microscopy [59], laser scanner [60], Surface-Enhanced Raman spectroscopy (SERS) [61, 62]; whose methods can be also classified as optical methods. Other well-known methods use enzymatic polymerization [63], biofunctionalized ion-sensitive field-effect transistors [64] and varieties of methods based on different imaging systems [65]. The biomolecules with corresponding tags as enzymatic,

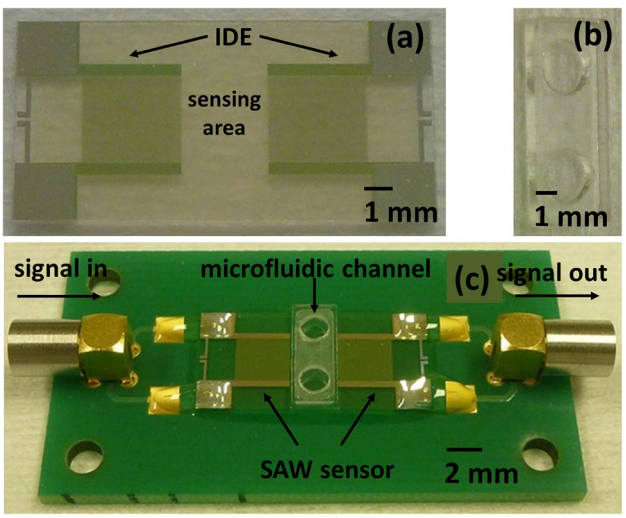

Fig. 3 SAW biomolecules detection system [57]: **a** SAW sensor quartz die with 2 pairs of IDE, **b** polycarbonate microfluidic system fabricated by micromilling, **c** fully functional biomedical detection system based on SAW sensor assembled on the PCB including two high frequency connectors for input and output signals

fluorescent, radioactive etc. bind to biofunctionalized layers. Then the corresponding target bound to specific biomolecules can be recognized using dedicated techniques.

Delivery System

The delivery system is intended to supply and/ or dose the test specimen to the sensing area of the biochip [66]. The delivery system can be very straightforward: for example, as mentioned in [67], the test specimen can be brought directly to the sensing area manually by a syringe (Fig. 4a) and the syringe will dose the material amount. In this case, an open cavity construction on top of the biochip acts as a simple fluidic system.

Depending on the volume (micro-, nano- or pico-liter) of the delivered material, the system can be micro-, nano- [68] or pico-fluidic [69]. Nowadays, a microliter range of specimen is the most common; therefore, the microfluidic systems are the most prevalently mentioned. The current trend [70] is to move away from fluidic to microfluidic systems [71], both to miniaturize the systems and to minimize the amount of specimen. The smaller the specimen quantity the better, as from a practical point of view, there is typically a limited amount of biological material available. Furthermore, the use of microfluidic systems results in faster analyzes due to the shorter reactions and delivery time of the specimen into the system.

Following the general trend for miniaturization, which also applies to advanced biomedical detection systems (Fig. 3b, [67]), the microfluidic system can be directly integrated on top of the biochip [72, 73]. A valuable alternative to that is the integration of the microfluidic system directly on the PCB [74, 75], that can in its turn result in an overall system cost reduction. Typically, the microfluidic system includes microfluidic channels, (a) chamber(s), often called (a) reactor(s), and inlets and outlets that connect the system to the outside world. The microfluidic system serving as an advanced delivery system can be equipped with a variety of components such micropumps, microvalves, flow and pressure sensors that can be incorporated inside the microfluidic system or can be integrated as discrete components [76].

The microfluidic system can comprise additional functionalities such as sophisticated system of microchannels for enabling a purge option. This feature enables the purging of the system after each testing cycle.

It can also comprise a reactor chamber that can be equipped with different analyzers and transducers such as ultra-sonic transducers [77], optical [78], electrophoresis features [79] and X-ray absorption spectroscopy analyzers [80]. Additionally, it can perform some specific functions such droplet forming and merging, filtering, mixing and even performing chemical reactions. To control such reactions, thermal management features, such as heating [81] and cooling, can be integrated. An interesting approach to manage the volumetric flow rate of the liquid pumped in the microchannel is presented in [82]. The flow rate depends on the characteristics of the micropillar array (height, diameter and pitch) integrated in the microchannel. The authors studied the effect of a microarray of pillars (Fig. 5a) in the microfluidic channel. They demonstrated that depending on the pillar geometry it is possible to facilitate or slow down a liquid flow though the corresponding microchannel.

The experiment shows (Fig. 5b) that specific pillar geometry as $h = 80$ µm (same as the channel height) and pillars of diameter $d = 400$ µm distributed with a pitch $p = 800$ µm can create a capillary flow that results in a

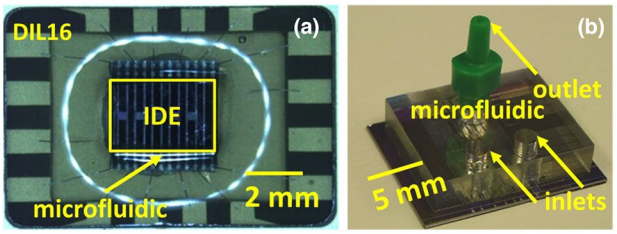

Fig. 4 Biomolecule detection system with microfluidic system [67], **a** an open cavity microfluidic system of volume of 1–1.5 µL range, with 3 mm long, 1 mm width, and 0.5 mm deep. Assembled in a dual in-line (DIL 16) ceramic package; **b** a differential microfluidic system with two microfluidic channels of 300 µm width. The microfluidic system is integrated with a silicon biosensor die with aluminum IDE

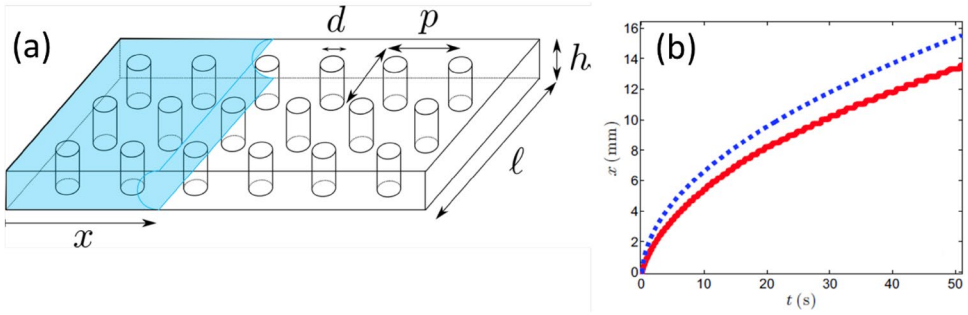

Fig. 5 **a** Sketch of the microarray of pillars, where h is a channel height and ℓ is its width. The diameter of the pillars is d, the pillars spaced by a pitch p. The height of the pillars is to the height of the channel. X is the position of the contact line. **b** Position of the contact line x as a function of time t

significant increase in the propagation of the contact line (X) comparing to a geometry with no micropillars.

Materials Consideration

In the Section "Biosensing system", we have defined biomedical systems as consisting of three main functional parts: the biochip, the microfluidic system and the read-out electronics. The first two parts, namely the biochip and the microfluidic system come in direct contact with the analyzing biomaterial. Meanwhile, the read-out electronics doesn't have the direct contact with the latter. Additionally to the biochip and the microfluidic system, an interconnect material (such as an adhesive) can be present to assemble permanently or temporarily these two parts. In this section we will present the materials that are typically used for the biochip, the microfluidic system and the interconnect material. Typically, a biomaterial is defined as a material that has been developed to interact with any type of biological material. According to Nature Portfolio definition "biomedical materials are biomaterials that are manufactured or processed to be suitable for use as medical devices" [83]. When biomedical materials come in direct contact with the analyzing biomaterials they must have a required level on the biocompatibility. Biocompatibility is defined by The Williams Dictionary of Biomaterials [84] as "the ability of a material to perform with an appropriate host response in a specific application". In the given specific case of the biomedical system for the biomolecules detection, we define biocompatibility as not causing any unwanted response or reaction to the tested specific biomaterial.

Guidelines to test biocompatibility are defined by the international standard (ISO 10993) [85] that is updated every 5 years. The objective of the biocompatibility testing is to determine the toxicity of a material and its effect during its operational conditions (such as chemical and physical), in various environments. The standard ISO 10993 defines the devices' categories based on the nature and duration of the contact regime with the biomaterial. Similar to the international standard (ISO 10993), the USA adopted the United States Pharmacopoeia IV (USP Class IV) Biological Reactivity Test [86].

Some practical recommendation on the biocompatibility testing for biomedical materials can be found in [87].

Despite a global harmonization of the definition of a medical device, there are numerous regulatory bodies worldwide that govern the certification of medical devices, from which we will mention the FDA (USA) [88] and the Regulation (EU) 2017/746 (IVDR) of in vitro diagnostic medical devices (IVD) [89].

Additionally to the required level of biocompatibility, the material used must also be compatible with the specific chemicals and solvents typically used in life science.

Biochip Materials

Single crystal silicon, which is the most commonly used material for large-scale manufacturing in the semiconductors industry, is also used for biochip manufacturing. Among varieties of manufacturing platforms, CMOS [90] and silicon MEMS [91] technologies are the most mature and have the largest market share. Silicon-based technologies use Si, silicon oxide (SiO_2), silicon nitride (Si_3N_4), silicon oxynitride (SO_XN_Y), silicon carbide (SiC), polycrystalline silicon and thin metal films [92]. Silicon-based materials are inert and cause no unwanted response to biomaterials. Some metals, for example Ti, Au, Pt, Ir fulfill basic requirements for the biocompatibility. Other metals such W, Cr, Cu, Ni or Al must be confined by layers of biocompatible material to exclude a direct contact between them and the analyte. Typically, W is covered by Ti, Cu by Ni that act as a diffusion barrier, then Ni is subsequently covered by a thin layer of Au. If Al material is used for the IDE, it is confined by an inert thin layer (typically 50 nm) of SiO_2, for example.

Among other important non-organic materials that are widely used for biochip manufacturing one must mention traditional microelectronic materials such as glass, quartz and ceramic. The thin-film processing technologies on glass and quartz present a wide degree of similarities with Si technology. They are also known for MEMS manufacturing and are a mature technology. The thick-film processing on ceramic has its specificities that clearly distinguish it from Si technology. The ceramic processing is based on screen-printing technology. Each functional layer is deposited using the corresponding stencil. Typically these layers are 10 µm and thicker, and have a widths of at least 100 µm. That is significantly larger that can be achieved by silicon and MEMS processing. The main advantage is lower cost compared to silicone-based technologies. The most common ceramic material are alumina (AL_2O_3) and zirconia (ZrO_2) ceramic, some of them have already been tested to different biocompatibility levels and marketed as a bioceramic or biomedical ceramic.

Following the cost reduction pressure, biochips can also be processed on a variety of organic materials, such printed circuit boards (PCB), which in its turn can be rigid or flexible. Other common materials are PDMS, PMMA, structural resists such KMPR, SU8 and conductive polymers [93]. The main difference with silicon-based and MEMS technologies is that such technologies are less accurate and, as result of that, comprise larger pattern features. For example, in case of IDE processed using a silicon-based technology, the lines and spaces widths are typically a few µm. Meanwhile,

such features are significantly larger, at least in the tens of microns range, if processed using PCB based manufacturing technologies, as by laser ablation for example [94, 95].

Such materials are often used for microfluidic systems manufacturing. We will discuss their use for such function in detail in the corresponding section.

To finish this section we will mention an increasing use of modern and intelligent materials such nanomaterials [96], graphene [97], that open new perspectives in biosensors functionality and sensitivity.

The nanoparticles have an advantage compared to micro particles widely used for biodetection, as they have a smaller size and a larger surface-to-volume ratio. That can benefit [96] for biomolecule labeling, bioimaging, drug delivery etc.

Graphene, because of its unique physical and chemical properties, opens new opportunities for biodetection applications [97, 98]. Graphene typically [98] supports the incorporation of different biological molecules and can serve as transducers of a biological signal.

However, there is an increasing awareness and concern of the effects of nanomaterials on the environment and health, which requires the setup of a risk assessment [99].

Microfluidic System: Materials and Manufacturing

The microfluidic system comes in direct contact with the tested biological material. Therefore, additionally to chemical, mechanical and fluidic properties, the microfluidic system materials must meet specific requirement for the biocompatibility. As described in Section "Materials consideration", the corresponding materials must be tested accordingly to meet specific levels of biocompatibility.

The materials can be classified in two main categories: organic and non-organic materials. Most microfluidic systems are made from organic materials due to cost and manufacturability considerations. Among non-organic materials, it is worthwhile to mention silicon and glass, as they are used for specific purposes.

The organic materials are usually available in solid or liquid form. Depending on the latter, the microfabrication method will differ. The solid form material can be processed by milling, laser ablation, thermal forming, 3D shaping etc. The liquid form can be processed using a variety of methods such as spinning, casting, molding etc. They can have several modes of polymerization: heat, UV, humidity etc.

The right choice of materials and manufacturing methods, in addition to the properties of the material, also depends on the volume of production and the cost model.

The manufacturing methods used for microfluidic system fabrication differ based on the production scale, and can be classified as for large-, medium- and small-volume productions. It should be observed that some methods can be also suitable for different production scales, leading to a possible overlap between the production methods.

Special interest lies in technology and methods for low-volume production that are widely used for device prototyping and proof-of-concept fabrication. For this application, the direct cost of material and manufacturing is less critical. The most important parameter is the development time necessary to obtain the prototype that will be used to demonstrate the concept. The low-volume production methods are widely used by research organizations and spin-off companies. Once the concept is demonstrated, alternative technologies which are more suitable for larger production scales can be deployed. These technologies usually optimize material use in terms of cost and performance.

Typically, manufacturing technologies such as hot embossing, injection molding and extrusion are used for large- and medium-volume production. Micromachining by mechanical micromilling and laser ablation technologies is for small-volume manufacturing. Lithography-based micromachining techniques originated from semiconductor manufacturing, specifically from CMOS and MEMS processing, are widely used for microfluidic systems for medium- to large-volume manufacturing.

The most common biocompatible plastics used for large-scale manufacturing of biomedical devices [100] such as biochips and microfluidics systems are cyclic olefin copolymer (COC) and cyclic olefin polymer (COP), polyetheretherketone (PEEK), polycarbonate (PC), polyvinylchloride (PVC), polymethyl methacrylate (PMMA), polyethersulfone (PES), polyethylene (PE), polyetherimide (PEI), polypropylene (PP) and liquid crystal polymer (LCP) [101]. The biocompatible plastics are divided in two main categories: thermoplastic and thermoset polymers.

The thermoset polymers are cross-linked, they have a high thermal resistance and they are typically mechanically strong and rigid. The thermoplastic polymers can, in theory, be remolten an unlimited number of times, which makes them easy to recycle. In practice, to improve their mechanical properties and increase their thermal resistance, they contain chemical additives. These additives typically strengthen the bonds and limit their potential recyclability. Many of biocompatible polymers are thermoplastics, such as PMMA, PC, PI, PU, PE and PS.

The corresponding properties of such material are extensively studied. For example, a summary of their thermomechanical properties can be found in [102]. Their chemical resistance is also tested and summarized in [102]. Generally speaking, they are chemically compatible with most of the solvents and other chemical products commonly used in life science and biomedical detection.

Most of them are suitable for hot embossing, injection molding and extrusion [101]. Some of them can be micromachined (by mechanical micromilling or using a

laser), which makes them suitable for medium or low scale manufacturing, for proof of concept designs or for niche applications.

One of the most common materials for microfluidic channel fabrication for low scale manufacturing and prototyping is PDMS [103]. Often it is applied by casting onto the pre-fabricated master, and subsequently cured. Typically, PDMS is a two components polymer: a pre-polymer and a cure agent. Prior to their use, they are mixed together according to manufacturer recipe. The curing schedule can vary and depends on the specific material or other constraints. The curing time can be as long as 48 h at room temperature and as short as 10 min at 150 °C. Some PDMS can contain an UV cure agent which allows them to be fully cured under intensive UV light illumination within several seconds. The main drawbacks of PDMS are the relatively high cost of raw material and unwanted contaminations that are difficult to eliminate. For instance, siloxane fragments transfers from PDMS on surfaces and causes contamination. The corresponding contamination is difficult to remove using conventional methods and it continues to deteriorate the surface.

Recently, Mercene Labs AB, developed family of products under the trade name OSTEMER [104] specifically for microfluidics, lab-on-chip assembly and organ-on-a-chip [105]. OSTEMER epoxy-based resins are supposed to overcome the main drawbacks of PDMS, namely contamination and low adhesion, while being applied by casting in a very similar way to the PDMS process. The process flow for casting and bonding a molded OSTEMER workpiece is illustrated in Fig. 6.

As reported in [104], the OSTEMER resin is resistant to most common chemistry used in life science such as toluene, acetone, dimethyl sulfoxide (DMSO), ethanol and methanol. Interestingly, according to the manufacturer, it has a minimal absorption of small molecules. It is an optically clear material, with low fluorescence. Mechanical properties of OSTEMER cured resins vary from stiff to flexible.

Another large family of materials that are used for low and medium scale manufacturing are structural resists, available in liquid form. Because of their application mode by spin-coating, they are often called as spin-on materials. After spinning, they are sequentially patterned to form the required microfluidics features. Most of them are photosensitive materials and can be manufactured using various lithography-based methods.

The lithography-based method is derived from earlier semiconductor technology that was also used for CMOS manufacturing. It is based on using a photoresist that is applied on the substrate by spin-coating. Depending on the viscosity of the photoresist, it forms a layer with a thickness between 0.2 and 5 µm [106]. Later on, following the demand for thicker layers (10 -100 µm) required for MEMS fabrication [107] and above-IC integration on various platforms, more versatile and thicker structural resists were developed. The thicker layer is achieved using higher viscosity resists, a different chemistry and sequential layer-by-layer deposition.

The typical microfluidic channel width range is between 20 and 1000 µm, the most common lying between 100 and 200 µm with aspect ratios of 1:1 or 1:2 (channel width to wall thickness) [108]. This was achieved by further composition modification of existing spin-on structural resists, and adopting previously developed material used for casting through spin-coating, such as PDMS for example.

Currently, the most common structural resists used for microfluidic manufacturing are SU-8 [109], KMPR [110], PI, parylene and spinnable version of PDMS and OSTEMER. Most of them are photo-patternable, which enables the use of lithography-based processing [111] to create the microfluidic system, including microchannels of variable widths and other features such as reactors, chambers and etc. In case of non-photopatternable structural resists, they can be patterned using micromachining or laser ablation.

The main advantage of spin-on resists is that they can be processed on silicon and glass wafers (starting from 3–4 “up

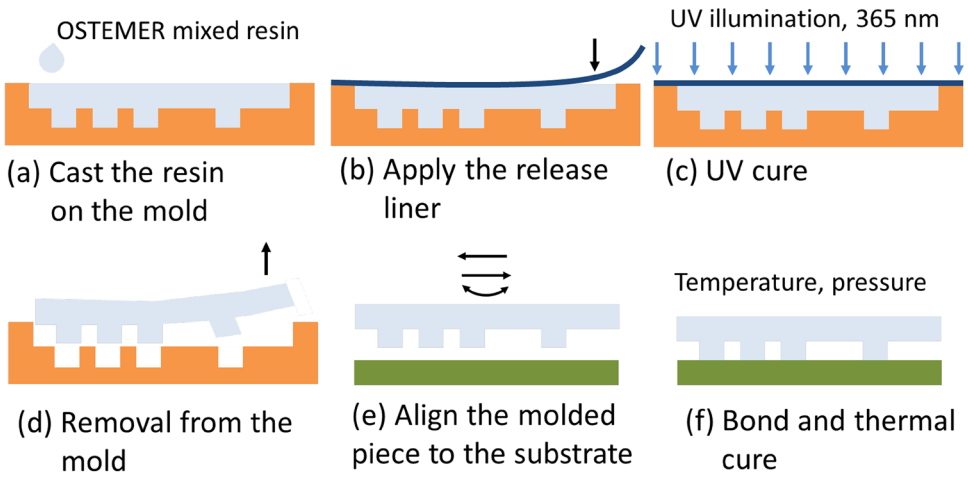

Fig. 6 Typical process flow for casting an OSTEMER workpiece and sequential permanent bonding to the substrate

to 8 and 12" diameter) by the lithography-based microfabrication technology. Such technologies are mature and can be easily ramped-up for large scale production.

Another rapidly emerging technology is 3D printing [112]. It is used for processing different parts including a delivery system for biomedical devices. The 3D printing technology opens opportunities for using unconventional materials, with a special interest among them for biodegradable plastics [113] and their recyclable alternatives [114].

In [115] authors demonstrated the direct-write microfluidic fabrication process that is conceptually simple and comprises several processing steps. The first step is dispensing the sacrificial layer. An epoxy resin is then applied on top of the sacrificial layer and sequentially cured. Finally, the sacrificial layer is removed using heat and vacuum. With such method, it is possible to obtain a minimal channel width between 100 and 200 µm, depending on the dispensing method utilized.

Researchers are constantly looking for straightforward and rapid alternatives to the lithography-based manufacturing technology and, following a demand for low-cost production, examine microfabrication methods that do not require the need for cleanroom environments. In addition to the micromachining method presented above, [116] reported on the suitability of other microfabrication methods such Print and Peel methods (PAP), laser jet, solid ink and cutting plotters.

Assembly and Integration Methods

In the Section "Biosensing system", we have identified the most common architecture of such systems. This architecture comprises three main functional parts that are typically processed separately. In a further stage of processing, the biochip and microfluidic systems must be integrated into a fully functional system. We also reviewed numerous biochips currently used for the detection of biomolecules. Because of several varieties of biochips, there is until now no common platform for the biochips integration. Each type of biosensors must be integrated differently and have its own integration specificity. In this section, we introduce the most common approaches.

The standard microassembly and integration technology [117], often called IC packaging technology in the literature, was developed for non-organic materials such Si, Ge, polycrystalline Si, SiC and others. The IC packaging technology includes several assembly steps, during which the assembly parts are subjected to different type of chemical and/or physical exposure. It starts from an exposure to water flow under high pressure with a combination of dust particles during the wafer singulation process, done by sawing. A thermal exposure up to 150 °C for a period up to 2 h is very common during several assembly steps such as die attach and adhesive curing. The interconnect technologies such as wire bonding, flip chip and soldering methods require also high temperature and force. Additionally to that, other impacts such as ultra-sonic (US) waves and ultraviolet (UV) irradiation might potentially cause damaging effects.

In the case of MEMS packaging and integration [118], the overall processing temperature must be reduced [119] to typically 150 °C in order not to damage sensitive parts of the MEMS devices. These are typically suspended parts such as beams, bridges etc. In order to further protect these sensitive features, they remain attached to the device during the most critical steps of processing such as, for example, during the wafer singulation by sawing and during high temperature processing steps. This prevents any unwanted movement and possible damage. Such MEMS are called unreleased MEMS. Once the MEMS underwent the most critical processing steps, the support for suspended parts can be removed by the so-called MEMS release process.

In case of a biochip with an already applied biofunctional layer, the biofunctionalization will likely be severally damaged during singulation by sawing.

Another important issue is a thermal resistance of biomolecules included in the biofunctionalization layer. The safest temperature range for comfortable existence of biomolecules lies in a range between 20 and 40 °C [120], which allows biomolecules such as proteins and nucleic acids to perform enzymatic activities, which is necessary for the normal function of living organisms. Typically, at temperatures above 40 °C, any kind of instability or degradative reactions can occur in molecules However, most of them can survive a temperature rise above 85 °C for a short duration [120]. Work [121] analyzed the stability of small biomolecules as a function of the temperature, and demonstrated that some molecules can withstand temperatures of 150–180 °C. From a practical point, to define the thermal resistance of a specific biomolecule, it must be tested accordingly and individually in the corresponding medium.

UV exposure can also in principle compromise the biofunctionalization. Each dose of the UV exposure must be tested on specific biomolecules. Meanwhile, the effects of UV wavelengths were not comprehensively studied yet, as there is no record that these have a significant effect.

The simplest way to overcome this issue is to develop an integration method where the biofunctionalization is performed as the last step of the assembly, although it is often not possible at all or not cost effective. In such methods where the biofunctional layer is applied in the last processing step, it can be done on each individual (fully or partially assembled) biochip. However, the biofunctionalization at the wafer-level where all biochips can be treated simultaneously as a high throughput collective process remains the primary goal.

If the biofunctionalization can be performed as a last step of the integration, there is still an important remaining issue to be solved, namely to reduce the overall thermal exposure during the first steps of the assembly. The biochip, according to the material it is made of, and the microfluidic system might indeed also be sensitive to high temperature exposure, unlike IC or MEMS devices that are made of non-organic materials and can withstand a high temperature and prolonged thermal exposure.

The process flow for integration of a microfluidic system with a biochip is conceptually straightforward and comprises several steps such adhesive application, alignment, bonding and curing (Fig. 7).

In Fig. 7 we presented the most common variation of this integration scheme. Often, the integration requires an adhesive to provide a permanent bonding of the microfluidic system to the biochip. Such adhesives, in addition to the usual criteria such as providing a set mechanical integrity, must fulfill some additional ones. The first requirement is the biocompatibility if the adhesive comes in a direct contact with a test specimen. Generally speaking, there are a number of adhesives that are proven to be biocompatible to different biocompatibility levels. Among them, the most commonly used for permanent bonding of microfluidic systems to a biochip are: polyurethane- and epoxy-based adhesives, varieties of epoxy-polyurethane blends, cyanoacrylates and PDMS or silicones. Another important requirement is the low cure temperature, to avoid any possible thermal damage to the assembly parts. In case of the presence of biofunctionalization, the cure temperature should not be higher than 40 °C, and should preferably be the room temperature. The adhesive manufacturers offer solutions such UV-curable adhesives or two components adhesives. For example, polyurethane-based adhesives from Norland [122] require relatively low UV (wavelength of 320–380 nm) energy densities of 3–4.5 J/cm^2.

Practically it takes between 5 and 30 s to fully cure the adhesive layer, depending on the layer thickness. The UV light is highly concentrated and applied locally, causing no damage to the biofunctional layer. The acrylated urethane adhesive formulated by Henkel [123] can be fully cured not only by UV light, but also by visible light with high energy density. The cure time varies from 2 to 30 s and depends on the adhesive thickness and the light source wavelength.

The second solution is to use two components adhesives [124] consisting of a primary resin and curing agent (or binder or hardener [125]. For example, Master Bond [124] offers two component epoxies and silicones adhesives. Dow [126] offers a broad range of two components silicones adhesives.

EPO-TEK offers [127] a "MED" line of biocompatible/medical grade adhesives, many of them being UV curable. As some of the Norland [122] and Dymax [128] products, they can be additionally thermally cured to achieve better performance, typically improved adhesion, mechanical and/or chemical properties.

Biocompatible cyanoacrylates [124] cure within minutes but their main disadvantage is a short self-life. Once the container is open, it hardens within days, while maximum shelf life of 12 months is obtained if the original and unopened containers are stored at 1.5–4.5 °C [124].

In some cases where the materials to bond are identical, an adhesive might not be required. For example, if a biochip is processed on PDMS (or has PDMS finishing layer) and

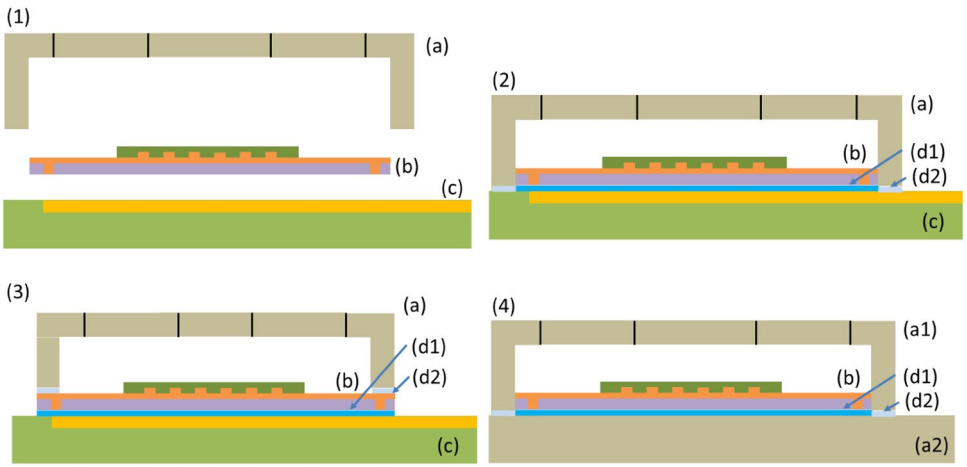

Fig. 7 An integration process of a microfluidic system and a biochip into a fully functional biomedical system. **1** The microfluidic system (a), the biochip (b) and the receiving substrate or carrier (c). **2** The biochip mounted on the substrate [using an adhesive (d1)], then microfluidic system mounted on the substrate [using an adhesive (d2)]. **3** The microfluidic system mounted on the biochip [using the adhesive (d2)]. **4** The biochip mounted on the bottom part of the microfluidic system (using the adhesive (d1)], then microfluidic system mounted on the bottom part of the microfluidic system [using the adhesive (d2) or without adhesive)

the microfluidic system is also made with PDMS a PDMS to PDMS bonding can take place [129]. This process is well studied and characterized. Usually, not fully cured PDMS parts can be co-cured together and form a joint [130]. Similarly to that, it is straightforward to bond OSTEMER to OSTEMER [131]. To conclude this paragraph, one can state that bonding similar materials or materials with similar chemical compositions is often possible without an adhesive, whereas bonding different materials usually requires an adhesive layer.

The most important requirement to achieve a good adhesion between surfaces is the compatibility of the joining materials, as well as the cleanliness of the surface and proper physical conditions. A surface treatment can be performed to improve adhesion. Typical treatment may require solvents and plasma cleaning or plasma activation. Because of the biocompatibility requirements, the range of possible surface treatments is limited.

Table 1 summarizes [117, 118, 125, 132] compatibility between the different biocompatible adhesives (epoxies, silicones, polyurethanes and cyanoacrylates) and the most common materials used for biomedical applications in function of bond strength.

The data given in Table 1 corresponds to the bond strength achieved without using surface preparation or treatment. The best adhesion is achieved using epoxies and cyanoacrylates on all materials. Generally, polyurethanes present a good adhesion to non-organic material and a fair adhesion to plastic and resins, hence these characteristics can vary according to specific materials. A specific attention must be paid to silicone adhesives. On some plastics, it is not possible to achieve long lasting and strong joints, while other surfaces require a specific preparation.

To illustrate Table 1, two different materials as PMMA and PC can be glued together using UV-curable adhesives; both of them are transparent to the UV light.

If bonding PDMS to PDMS is relatively easy and a well-known process, bonding PDMS to plastic and resin materials is challenging and the joining surface must be prepared. The preparation can include oxygen plasma treatment followed by silanization [130]. Even after silanization, it is not always possible to obtain the required bond strength and researchers are looking for additional measures, such as in [130] where the use of a n UV curable adhesive is reported.

Table 1 Bond strength between the different chemistry adhesives and the most common material used for biomedical applications

Surface	Adhesive chemistry			
	Epoxies	Silicones	Polyurethanes	Cyanoacrylates
Metals	Excellent	Fair	Good	Excellent
Silicon	Excellent	Fair	Good	Excellent
Glass	Very good	Fair	Good	Very good
Plastics	Very good	Poor	Fair	Very good
Resins	Very good	Poor	Fair	Good

Once the adhesive is applied, the microfluidic system must be aligned with a required accuracy towards the biochip. For that, a die bonder is used. Typically, the alignment accuracy provided by the die bonder is better than 25 µm and is sufficient for the required application. Once the alignment is performed, the bond head presses the microfluidic system against the biochip with a designated force. A die bonder is capable to apply a broad range of force. However, in case of adhesive bonding, the force is typically low, in the range of few Newton. Once the microfluidic system is mounted, the adhesive curing is performed using one of the aforementioned methods: UV exposure or thermal curing. In the assembly flow (Fig. 7), we illustrated the mounting of the microfluidic system to the biochip. While this assembly flow may be used for mounting a biochip to a microfluidic system, and assembly microfluidic system that made of two parts, a base part and the cover part of the system.

After mounting and permanent fixation of the microfluidic system to the biochip, the obtained system must meet minimal requirements for mechanical integrity. The joint between the biochip and the microfluidic system must provide sufficient shear strength. To the authors' knowledge, there is no standard that defines a minimal shear strength for such applications. Stoukatch et al. [57] reports a standard that defines a minimal shear force for an IC mounted on the substrate, Die Shear, MIL-STD-883 F, Method 2019.7 [133].

Additionally to the adequate mechanical robustness, the obtained seal must meet a minimal leakage tightness specification. Indeed, the joint between the biochip and the microfluidic system must form a reliable seal that has no leakage during specimen analysis. There are two different levels of leakage tightness, namely gross- and fine- leakage tightness. The system must, at least, be gross-leakage tight. In order to validate this [57, 67] (as illustrated in Fig. 8) a red ink is injected and the sealing joint is observed under a microscope.

Bhagat et al. [134] reports on the use of a re-usable compression-based fluidic connection to detect any leakage in the microfluidic system: it also uses a high-pressure test up to 1.7 MPa for the characterization of the mechanical integrity of the system.

Typically, microfluidic systems have no specific requirements for fine-leakage tightness.

Conclusions and Outlook

The current technological drivers for detection systems targeting biomolecules is to perform rapid analyses, to use less test material and to be more accurate and selective.

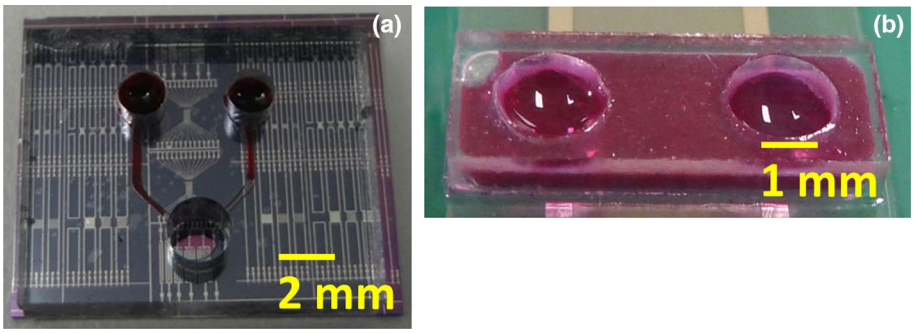

Fig. 8 The gross-leakage tight test demonstration. The red ink injected in the inlet of the microfluidic channel: **a** a microfluidic system bonded on a silicon biochip, **b** a microfluidic system bonded on SAW quartz chip

Typically, different types of biomolecules must be detected and analyzed. The technology development is tightly paired with a constantly increasing pressure for cost reduction, miniaturization, and recyclability.

To fulfill such constantly increasing demands, the corresponding biomedical sensing systems are becoming more versatile and complex. The conventional detection methods such as enzyme antibodies, PCR assays and immunofluorescence microscopy have inherent limits to fulfill such requirements. Novel detection methods are rapidly emerging, such as impedance and magnetic biosensors, a large variety of optical sensors, SAW sensors and sensors based on other detection principles as well as a combination of known methods (e.g. a combination of SAW and optical sensing).

As the detection methods that employ label detection and biofunctionalization are still the most common and widespread, they remain more costly and labor intensive. In response to that, significant efforts are directed to develop a label-free detection techniques and development processes requiring no biofunctionalization.

The device processing technology that supports biomedical device manufacturing is also constantly evolving. The modern biochip fabrication does not only employ the most advanced CMOS and MEMS technology, but constantly looking for alternatives and using novel and smart materials such polymers, nanomaterials, graphene etc.

Special efforts are dedicated for the design of delivery systems that supply specimens to the sensing area. The most common delivery systems are microfluidic devices, which gradually evolve to nano- and pico-liter systems. Such systems require less test material, and are usually more accurate and provide results faster.

Meanwhile, manufacturing and integrating such fluidic systems with biochips to form fully functional biodetection systems becomes more challenging and leverages on the recent advances in microfabrication and integration technology.

Such challenges are dealt with using a multidisciplinary approach, including and merging knowledge not only from the traditional science involved, such as biology, biomolecular chemistry and biotechnology, but also including recent advances in material science, physics, chemistry and nano- and micro-technology and fabrication.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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