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Abstract. Microfluidic chips have become attractive devices with enormous potential for a wide range of applications. The optimal performances of microfluidic platforms cannot be achieved using a single fabrication technique. The method of obtaining the dominant characteristic of a microfluidic chip is to combine the best qualities of different technological processes and materials. In this paper, we propose a novel, cost-effective, hybrid microfluidic chip manufacturing technologies that combine 3D printing process and xurographic technique. The standard Y-mixer was 3D printed using thermoplastic polymers, while the enclosure of the channel was achieved using the PVC lamination foils. The influence of the fabrication parameters, materials and bonding layers on the channel dimensions, performances and durability in the process of chip realization have been analysed and tested. Optimized parameters have been established for 3D fabrication process. The potential application in biomedicine and material science has been demonstrated on the example with nickel-titanium (NiTi) orthodontic archwire.

Keywords: microfluidic fabrication; 3D printing, xurography, optimization, characterization, NiTi archwire

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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1 Introduction

Microfluidics has entered in biomedicine as an innovative discipline that combines manufacturing technology of microminiaturized devices and the physics of fluid behaviour on a sub-micron scale. Microfluidic devices could substitute and implement operations that require the expensive laboratory equipment, offering revolutionary new features for biomedical, microbiology, and microelectronics research and application (Sackamann et al. 2014; Yew et al. 2018; Shin et al. 2019; Pandey et al. 2018; Vidic et al. 2019; Shaegh et al. 2019). The possibility to implement different functions into one single microfluidic chip, such as DNA extraction, reagent storage, system for fluid mixture, particle separator, and/or electronic detection system, has dramatically increased the attractiveness of microfluidics-based devices. These devices have extended the microfluidic concept into complex lab-on-a-chip (LOC) system for point-of-the care (POC) diagnostics (Shin et al. 2019; Pandey et al. 2018; Vidic et al. 2019).

To address advanced requirements of the microfluidic devices ready for the point-of-care diagnostics, the research direction has been mainly focused on novel designs, advanced materials, and utilization of cutting-edge fabrication technologies for efficient prototyping and testing of the microfluidic system (Shaegh et al. 2019). The selection of microfluidic chip design, material for fabrication, and manufacturing technology depends on its application, complexity, operating temperature and pressure, durability, etc. Therefore, the current research in microfluidics is increasingly focused on the rapid fabrication processes and development of an inexpensive, biocompatible microfluidic chip with good performances that can be easily scaled up for mass production.

The three-dimensional (3D) printing as an additive fabrication technology and rapid prototyping technique can be used for manufacturing different microfluidic chip designs (Masood 2014). However, optical transparency, and deformation of the channels represent the inherent problems when this technique is solely applied (Duong and Chen 2019). This can be solved by incorporating glass or transparent thermoplastic materials such as poly-methyl-methacrylate (PMMA) or polystyrene (PS), and specific bonding process between layers. Incorporating glass can bring the advantages related to chemical stability and optical transparency, whereas polymers provide good mechanical characteristics (Chen et al. 2019). This implies that a combination of two or more techniques and/or materials can deliver better performance of microfluidic devices. Moreover, the tubing connectors can be easily embedded within microfluidic chip using PMMA or thermoplastic materials (Duong and Chen 2018). Different examples of hybrid technologies have been proposed such as, lightweight paper−plastic microfluidic chip for urine analysis (Jalal et al. 2017), nitrocellulose that combines cellulose acetate and PMMA for protein detection (Yuzan et al. 2019), and microfluidic platform based on acrylic-tape for biomedical testing (Ren et al. 2019).

Together with the improvement of microfluidic fabrication techniques, surfaced the xurography (Bartholomeusz 2006), lithography, and lasers enabling the use of devices with a smaller outline and reduced costs (Jayamohan et al. 2017; Kojic et al. 2018; Kojic et al. 2019). All these simple, economical, and fast fabrication methods have supported the application of various microfluidic setups into a wide range of biomedical areas. Nonetheless, POC diagnostics remains the main application area of microfluidics. The theranostics potential of POC devices sets the creation of a chip-based, self-comprehending scale down devices that can be employed for the examination of numerous, multiplexed analytes in complex substrates, such as saliva (Richard et al. 2016; Pulsipher et al. 2018) While there are intensive investigations regarding the prospect of a chip application, the reality is more like a chip in a lab rather than a lab-on-chip (Franca et al. 2019). Despite outstanding progress toward POC clinical systems, only a few completely functional prototypes have been developed (Connelly et al. 2015; Guckenberg et al. 2015; Nayak et al. 2017). A novel platform that enables investigation of the dental pulp response to various biomaterials was recently proposed (Franca et al. 2019). There are still important challenges in a creation of new salivary POC diagnostic devices in clinical practice, and the application of already designed setups for everyday use (Giannobile et al. 2011; Khan et al. 2017).

Nowadays, biomedical metallic devices are designed for use in various parts of the human body for medical or dental purposes. Once a material is placed either into the body or intraorally, a significant number of chemical reactions occurs on its surface. The widely used nickel-titanium (NiTi) alloys offer a number of benefits, but also pose a risk to human body. A major disadvantage of contemporary metallic biomaterials is limited lifespan due to friction, fouling, wear or decay of the material in warm, humid, and corrosive environment of the human body. The implantation or intraoral use of nickel titanium biomedical devices carries a risk of pathologic conditions linked to nickel release into oral cavity or tissue. Orthodontic NiTi wires and accessories are used intraorally. The wire is not implanted into the tissue but is placed into the oral cavity. Finding a way to measure and control nickel release from orthodontic wires in a highly corrosive environment, such as the human oral cavity, could give valuable information for biocompatibility management of implanted devices. Although the implantation procedure of biomedical devices may be considered more invasive than the intraoral placement of the Ni-containing alloys, the reactivity of the implanted material is low due to the formation of a connective tissue capsule surrounding the foreign body (Burkandt et al. 2011).

Intraorally placed materials such as wires or brackets exhibit a pattern of continuous reaction with the environmental factors present in the oral cavity. Such a continuous process is not the simple sum of all factors, but rather their reaction, which is not always predictable in size and type of corrosion and degradation. In addition, the variations in pH and temperatures, bacteria, medications, acid drinks, and chemoprophylactic agents, make nickel and other heavy metal release unpredictable. Therefore, design, manufacturing and optimization of microfluidic devices for use in material characterization and detection of heavy metal leaching have become one of the most important objectives in contemporary biomaterial science. This objective could be efficiently addressed with the establishment of a microfluidic setup developed with the intention for application in theranostics. The requirements include the specific design of numerous constituents comprising precise channel construction, sample preparation, selection of materials, control of mixing, combining and reacting. All these systems should contain functional, mechanical and electrical components, sensors and actuators.

In this paper, we propose two hybrid fabrication technologies that combine Fused Deposition Modelling (FDM) and xurography methods. Both processes are cost effective and allow rapid prototyping and fabrication of robust microfluidic chips with a good repeatability. The dimensions, performances, and durability of the realized microfluidic chips were examined, as well as optimization of the fabrication process. The prospective biomedical target of presented microfluidic chips would be the application in intraoral setting with fixed orthodontic appliance - orthodontic wire.

2 Materials and Methods

2.1 Technologies, materials, and equipment

The FDM is an additive manufacturing technology that provides affordable, fast prototyping, and fairly precise 3D models that may be used in microfluidic chip production (Yazdi et al. 2016; Bhattacharjee et al. 2016). Namely, FDM technology uses thermoplastic materials such as PLA (polylactic acid), TPU (thermoplastic polyurethane), and other, that in a direct contact with different fluids inside microfluidic channels will not impair the fluid characteristics or release different compounds into the fluids. With all its versatility, FDM technology may be greatly utilized in development of a new hybrid technology.

The xurography is a cost effective technique for microfluidics chip fabrication with a plotter cutter. The plotter cutter uses cutting blade which is traditionally used in the graphical engineering industry for cutting layouts in vinyl films with adhesives (Bartholomeusz 2006; Islam et al. 2015). Using this technique expensive and time-consuming process of photolithography may be altered with significantly simpler cutting process. In the recent period, xurography has been used in a wide range of applications from electronic passive components (Stojanovic et al. 2019), biology (Kojic, Stojanovic and Radonic 2019; Xiao et al. 2019) and various biomedical applications (Greer et al. 2007). The xurography technique usually uses PVC foils as a substrate as well as an active layer. Unavoidable part of xurography technique is a lamination process that is used to bind together adjacent PVC foil layers obtained from the plotter cutter.

Since both fabrication processes, FDM and xurography, offer good performances in microfluidic chip fabrication, combining both, it is a key enabler in utilizing the best of each technology while establishing the novel hybrid fabrication methods. The advance of fast rapid prototyping provided by FDM technology with ease of control of microfluidic chip dimensions and low-cost xurography technique represent two main properties of novel hybrid fabrication methods.

For the microfluidic chip fabrication by FDM technology the common thermoplastic materials have been used; PLA filament (by Devil Design, 2.85 mm diameter) and TPU filament (by NinjaTek Ninjaflex, 1.75 mm in diameter). The proposed filaments are commercially available materials, characterized by excellent printing properties. The filaments differ in mechanical properties, PLA is rigid and TPU is flexible. The different mechanical properties have been chosen in order to validate the adhesion characteristics during the lamination process with PVC foils. Since chosen filaments differ in diameter, two different 3D printers have been used: (a) the Ultimaker 2+ (UM) for PLA filament (2.85 mm in diameter), and (b) Felix Tec 4.1 (FT) for TPU Ninjaflex filament.

The layouts of the PVC foils as well as 3M double-sided adhesive tape have been created utilizing plotter cutter (CE6000-60 PLUS, Graphtec America, Inc., Irvine, CA, USA) with the 45° cutting blade (CB09U) and the cutting mat (12” Silhouette Cameo Cutting Mat, Sacramento, USA).

Following equipment have been used for the characterization purposes: Profilometer Huvitz Panasis (Huvitz BD, Gyeonggi-do, Republic of Korea) used for dimensional measurements of the inlets/outlets, observation field and microchannel dimensions. The cross-sectional measurements of chipsets were conducted with SEM micrograph, TM 3030 (Hitachi®, Tokyo, Japan). Experimental testing of the microfluidic chips was done with the syringe pump model NE 4000 Multi Pulser (KF Technology, Roma, Italy).

2.2 Hybrid technologies and parameter optimization

The layout of multi-layered microfluidic chips based on the proposed hybrid technologies is shown in Fig. 1. The design fabricated by the first hybrid technology, Fig. 1a, comprises of three-layer structure, bonded with a hot lamination process, whereas the design fabricated by the second hybrid technology, Fig. 1b, includes five-layer structure bonded with cold lamination process.

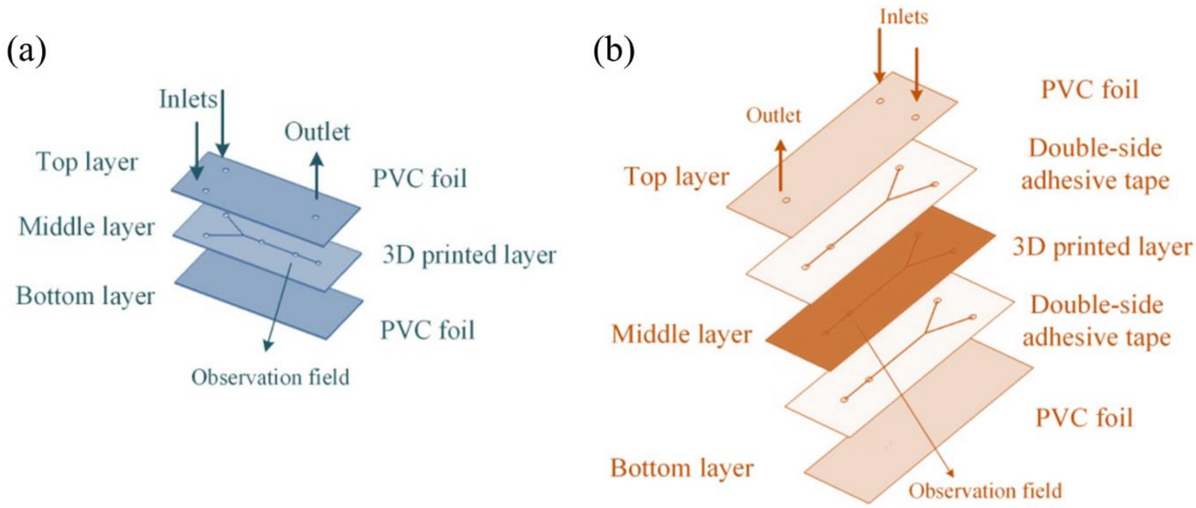

Fig. 1 Design of microfluidic chips for: **(a)** the first hybrid fabrication technology; **(b)** the second hybrid fabrication technology.

In the proposed hybrid technologies bottom and top layers were manufactured by xurographic technique using PVC foils with thickness of 125 µm. Top and bottom layers direct the fluid flow inside the microfluidic chip by enclosing the microchannel structure. The top layer contains holes for two inlets and one outlet, while the bottom layer closes the microchannel structure from the bottom side. The PVC foils were cut with the optimized parameters: cutting speed and cutting force, in order to get precise dimensions and even edges of each layer. The cutting parameters for PVC foil are given in Table 1.

Table 1. PVC foil cutting parameters

Parameter	Value
Cutting speed – inlet/outlet [cm/s]	30
Cutting speed – border [cm/s]	60
Cutting force	23[1]

[1]From the range 1 ÷ 38

The middle layer in the proposed hybrid technology was 3D printed using FDM. Namely, the middle layer in the first hybrid technology was fabricated using PLA filament, while in the second hybrid technology the middle layer was printed using TPU flexible filament. This layer was designed with Y-channel comprising of two inlet channels and straight-line microfluidic channel. The Y-channel with 60° angle has been selected as one of the most common designs used for passive micro mixing microfluidic testing (Nguyen and Wu 2004; Lee et al. 2011). The inlets were predicted to insert two different fluids into Y microfluidic channel, that would merge and continue flowing into a single straight microfluidic channel. The widening section in the straight microfluidic channel is used as an observation field, which is an area of the camera focus for recording the fluid properties during the experiment.

Since the printing parameters greatly influence the overall properties of the printed part, the most important 3D printing parameters were varied to optimize middle layer properties for further fabrication processes.

In order to obtain better adhesion during the lamination process, the bottom and top surfaces of 3D printed middle layers must be as smooth as possible. Namely, uneven surfaces may compromise the hot lamination process that can result in poor adhesion between middle layer and PVC foils from top and bottom side. Since the used 3D printers build platforms are overlaid with Kapton foil and/or borosilicate glass, the bottom surface of the 3D printed middle layers has satisfactory smoothness, while top surface demands higher smoothness. In order to reach satisfactory levels of smoothness in comparison to ordinary printed models, the ironing feature have been utilized in commercially available 3D slicing software, Ultimaker Cura. The ironing feature enables the hot end of the extruder to travel over the top printed layer with reduced extrusion to create the smooth top finish. The most important ironing settings were optimized: ironing line space, ironing speed, ironing flow, and ironing inset. Accordingly, other printing parameters such as print temperature, print speed and line width that have a well-known influence on overall printing quality were optimized along with the ironing feature.

After extended optimization process five sets of 3D printing settings have been obtained; three for the first hybrid method and two for the second hybrid method. These settings, provided the visually best overall printing results with the smoothest top surface finish of the middle layer, are given in Table 2.

Table 2. 3D printing parameters

3D printing settings	**Chipset 1**	**Chipset 2**	**Chipset 3**	**Chipset 4**	**Chipset 5**
Material	PLA	PLA	PLA	TPU	TPU
Print temperature [°C]	200	210	210	210	200
Print speed [mm/s]	40	20	30	20	15
Ironing speed [mm/s]	13.3	10	10	0.25	0.25
Layer height [mm]	0.1	0.1	0.1	35	25
Initial layer line width [%]	110	100	90	0.25	0.25
Ironing inset [mm]	0.25	0.2	0.25	20	15

A total of five chipsets each containing fifteen chips were fabricated. The first 3 chips from each chipset have been used for microscopical measurements and the rest of 12 chips have been used for microfluidic chip production due to variations of production parameters which will be explained in detail in what follows. The UM 3D printer was used for printing PLA middle layer for chipset 1, chipset 2 and chipset 3, while FT was used for printing TPU middle layer for chipset 4 and chipset 5.

In order to bring together the PVC foils and 3D printed middle layers the hot and cold lamination process were utilized. The hot lamination process was implemented for bonding the PVC foils with PLA middle layers, since at appropriate lamination temperature the satisfactory bond was obtained between these two materials. Four different temperatures and speed combinations tested are presented in Table 3, for treated chips in chipsets.

Table 3. Hot lamination parameters used in the first hybrid technology

Lamination	Temperature [°C]	Speed[1]
Combination 1	150	1
Combination 2	150	5
Combination 3	140	1
Combination 4	160	1

[1] From the range 1 ÷ 9

On the other hand, the second hybrid technology shows very weak bond while using hot lamination process to bring together TPU material and PVC foils. Therefore, in order to obtain strong bonding additional adhesive layers were added between middle and top, and middle and bottom layers, utilizing 3M double-sided adhesive tape in the cold lamination process. The thickness of adhesive tape was equal to 125 µm. In order to prevent the clogging of the channel during cold lamination process, the adhesive tape bonding layers were cut by plotter cutter with the same layout as the middle layer design. Using the 3M tape and cold lamination process, the adequate adhesion between layers was achieved for the second hybrid technology. The cutting parameters for 3M double-sided adhesive tape are given in Table 4.

Table 4. Hot lamination parameters used in the first hybrid technology

Xurography – 3M tape	All chipsets
Cutting speed – inlet/outlet [cm/s]	2
Cutting speed – border [cm/s]	2
Cutting speed – channel [cm/s]	2
Cutting force	20[1]

[1]From range 1 ÷ 38

It is important to note that exact dimensions of the microfluidic channel have been kept using the second hybrid technology, since the channel dimensions depend on 3D printing parameters and adhesive tape thickness. In contrast, consequences of the hot lamination process used in the first hybrid technology have had a slight deformation of the microfluidic channel caused by heat, which will be discussed in the next section.

3 *Fabrication results and measurements*

The middle layer layout of microfluidic chip designed in Libre CAD, and photographs of 3D printed middle layers fabricated in PLA and TPU material, are shown in Fig. 2(b) and (c), respectively.

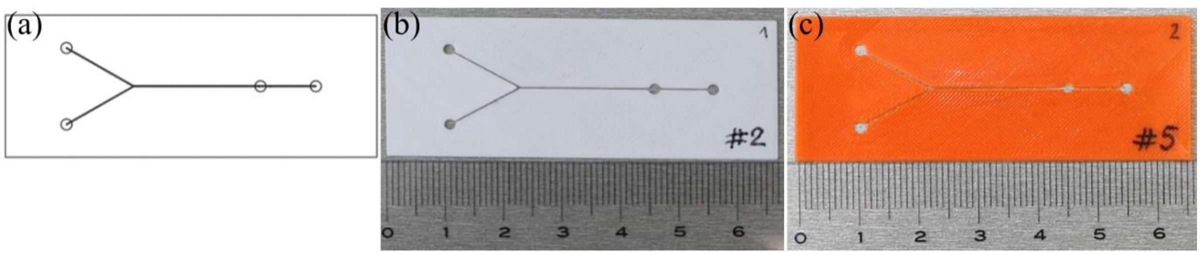

Fig. 2 The middle layer of microfluidic chip: (a) Libre CAD design, (b) 3D printed in PLA; (c) 3D printed in TPU (Ninjaflex)

The designed dimensions of inputs, outputs, and observation fields were set to 2 mm and the microchannel width was set to 200 µm. In order to determine degradation of dimension during lamination due to thermal sensitivity of the PLA material, the middle layer of all chips in chipsets 1, 2 and 3 were measured in detail before and after lamination. The middle layer of all chips in chipsets 4 and 5 were measured only before bonding, since no thermal procedure was used during lamination process.

For measurement purposes, fourteen measurement points were set for each chip in chipsets. Namely, eight measurement points for circular shapes in the design, two measurement points, to check the regularity of the circles, for each inlet, observation field, and outlet (Fig. 3 and Fig. 4a), and six measurement point on strategic places in the Y-microchannel, places on which possible deformations can occur due to printing and lamination fabrication processes (Fig. 3 and Fig. 4b). Although, SEM measurements are more accurate, we used an optical profiler for all measurements performed of the middle layer, because the testing after the lamination process was not possible using SEM due to non-conductivity of PVC.

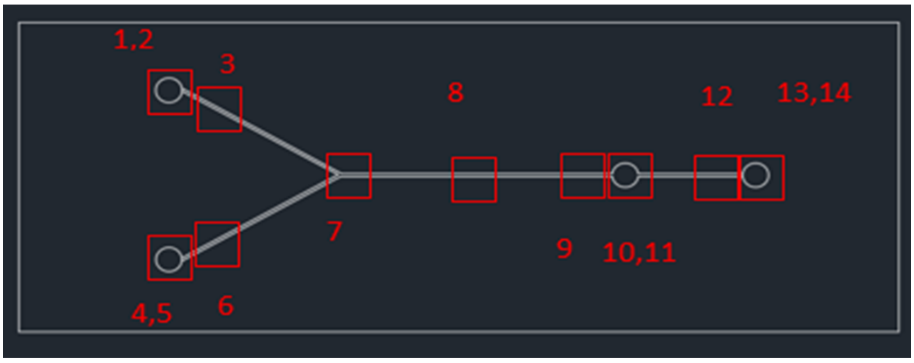

Fig. 3 Measurement points position

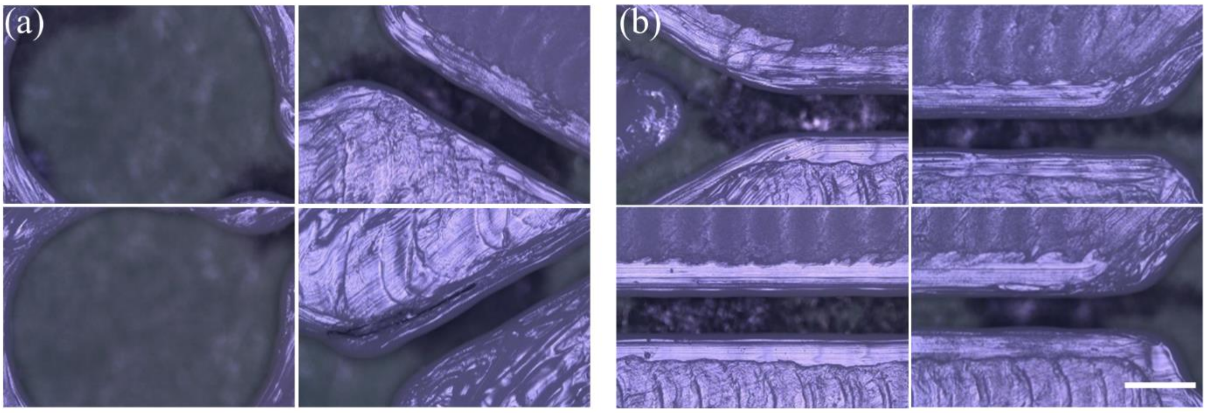

Fig. 4. Measurement points on an optical profiler: (a) inlets and (b) microchannels, with 500 µm bar depicted

In order to obtain precise information of the dimension and wall shape of the printed microchannel, chips were tested under SEM. The micrographs showed that microchannels of TPU layers were smoother than PLA layers, meaning they will cause less turbulence in the microfluidic flow. Moreover, TPU layers had more convex rectangular cross section of the microchannel, while PLA had more rectangular cross section (Fig. 5).

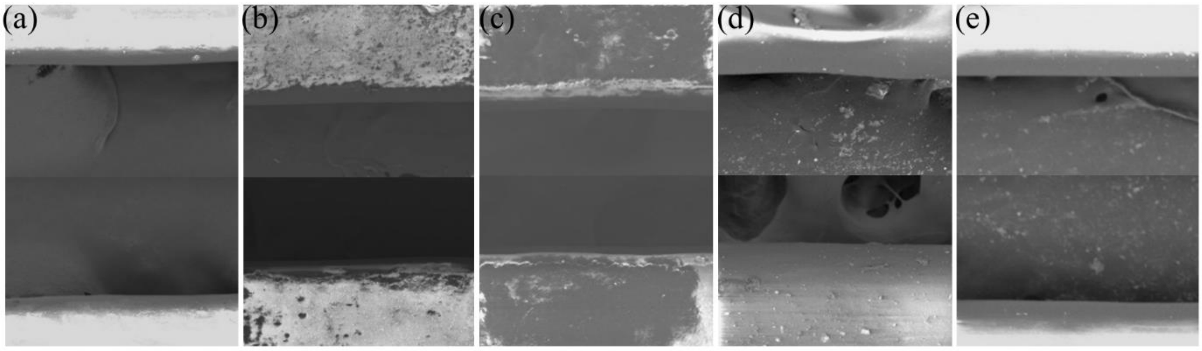

Fig. 5 SEM micrographs of microchannel edges on magnification ×500: (a) Chipset 1, (b) Chipset 2, (c) Chipset 3, (d) Chipset 4, (e) Chipset 5

All five chipsets were measured before the lamination processes, but for chipsets 1, 2 and 3 measurements were performed also after the hot lamination. Measurement after cold lamination process, for chipsets 4 and 5 were not performed since there was no thermal exposure, hence there was no deformation. A mean value, standard deviation, and dot plots of 14 measurement points per all 5 chipsets before lamination are demonstrated in Fig. 6. Although there is no apparent difference regarding the microchannels, it is obvious that fabricated circular structures for chipset1, 2 and 3 are more uniform than the same structures fabricated in TPU. Moreover, microchannel which for chipset 1 has been most accurate in the average, but with the highest standard deviation and some almost clogged channels. Further, chipsets 2 had have the best overall characteristics while chipset 3 had the microchannel much wider than designed, instead of 200 µm it has been around 350 µm. Concerning TPU middle layers, chipset 5 had wider channel with less narrowing when compared to chipset 4. This feature has been more desirable for future use.

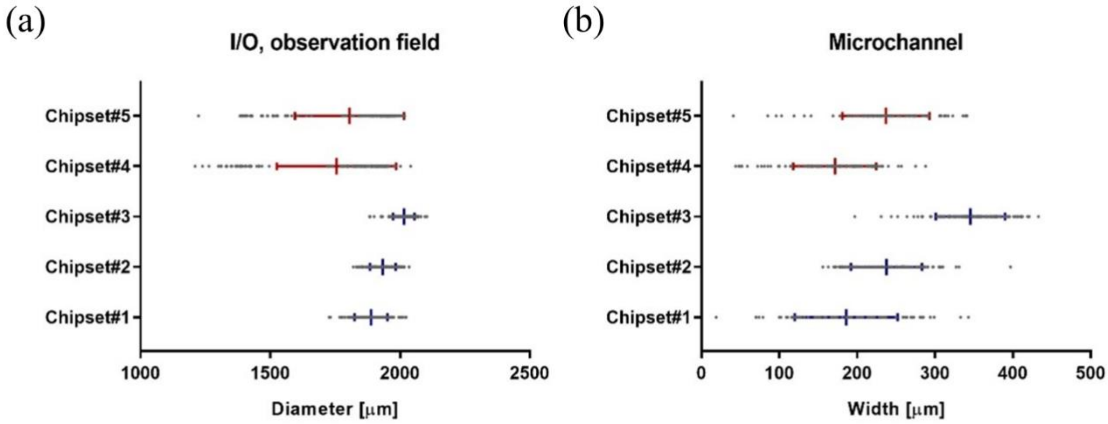

Fig. 6 Dimension of all chips before bonding, (a) Diameter for inputs/outputs (I/O) and observation fields – circular structures, (b) Microchannel widths – line structures

All statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). The normality of the data and the homogeneity of variance between groups were tested using the Shapiro-Wilks test. Since the data did not fulfil the assumptions of parametric data, the omnibus test was performed using a non-parametric equivalent of the repeated measures ANOVA, Friedman's repeated measures test was employed in difference significance interpretation. The difference between measurements before and after on circular shapes did not show a significant statistical difference on Friedman's test ($P = 0.327$, $P < 0.05$). However, the same test showed a significant difference in the dimensions of the microchannel (P = 0.0022, P < 0.05). Hence, the deformation of the design, especially of its critical parts, should be pre-calculated into the design.

Difference of dimensions measured before and after hot lamination, for chipsets 1, 2 and 3, with mean value and standard deviation, are given in Fig. 7. Chipset 1 has a very small degradation of dimensions for circular shaped design part, while microchannel went through significant degradation (narrowing). Some channels were almost clogged. Chipset 3 has opposite results; the channel is almost without the degradation while circular shapes have around 70 µm degradation. Chipset 2 is in the middle between Chipset 1 and 3. Concerning absolute dimensions shown in Fig. 6 and discussed before, the best fabrication parameters for creating a microfluidic chip in this technology are parameters for Chipset 2.

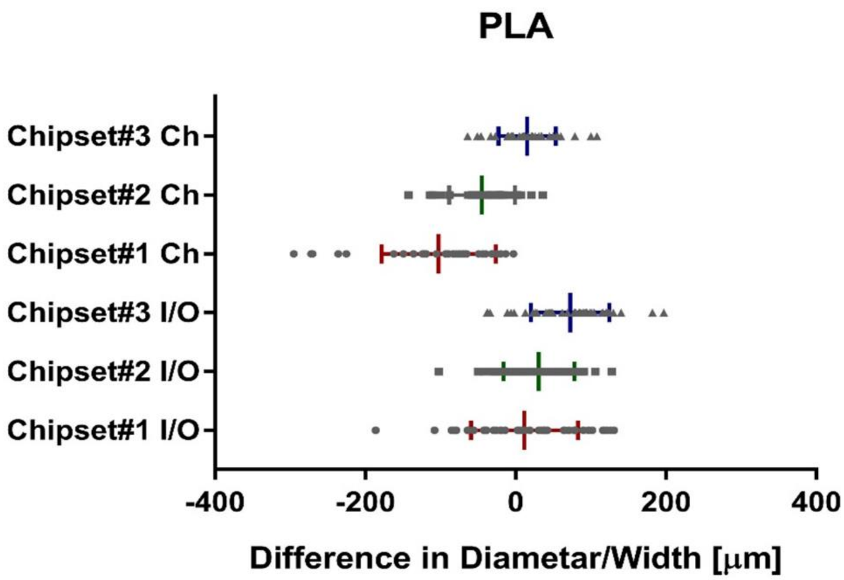

Fig. 7 Dimension differences for Chipset 1, 2 and 3, before and after lamination

3.1 Maximal flow rate

To establish the criteria for the highest possible flow rate through the chip, an experiment was set-up. Through all fabricated chips different flow rates were used until they started to leak. The experiment begun with the flow rate from 1 µL/m to 10 µL/min, with the step of 1 µL/min. The next range was from 10 µL/min to 100 µL/min, with a step of 10 µL/min. The third range was still in the microliter range, started from 100 µL/min to 1000 µL/min, with the step of 100 µL/min. The last range was in the milliliter range starting from 1 mL/min to 20 mL/min, with the step of 1 mL/min. All flow rates were kept stable for 120 s. Fig. 8 presents the review of flow rates that caused leakage of the fabricated microfluidic chips. The weakest point for chipsets with a middle layer, was the bonding of PVC foil to PLA. It can be seen in Fig. 8(a) that the maximum flow rate for chips made in the first hybrid technology can stand was around 3000 µL/min for Chipsets1 and 3, and for Chipset2 that value is 2000 µL/min. By comparing the parameters used for lamination from Table 3, the flow rate results show that the optimal parameters for lamination process are given by Combination 1 from Table 3. Chipsets with TPU middle layer bonded with 3M tape could stand much higher flow rates. It can be seen in Fig. 8(b) that strong connection between layers provides that chip can stand very high values of flow rates - maximum value of 11 mL/min in Chipset4 and 18 mL/min for Chipset5. In addition, Fig. 8(a) shows that all of the tested chips showed good durability performances and could stand at least 3 mL/min.

The weakest points of these chips were the adhesive pads (connection between the chip and the tubing), not the chip itself.

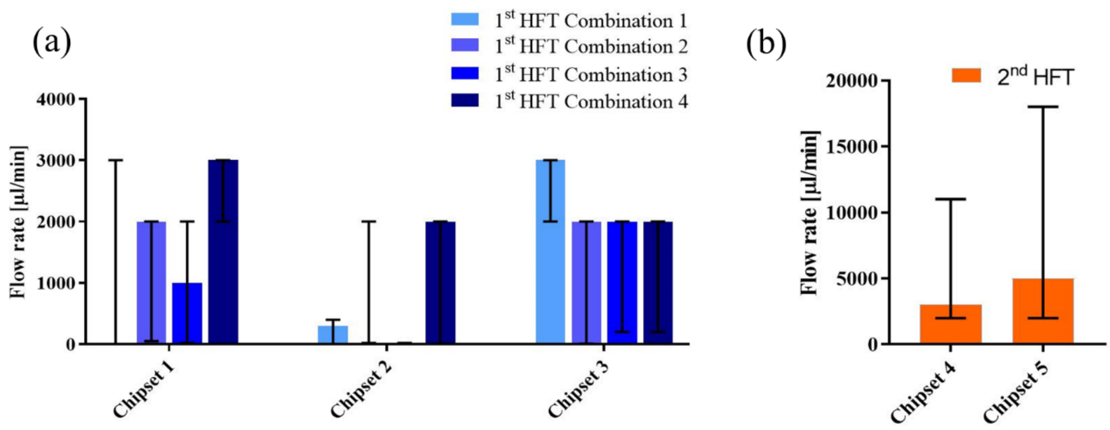

Fig. 8 Flow rate which caused chip leakage for: (a) Chipsets with PLA middle layer, (b) Chipsets with TPU middle layer (HFT – hybrid fabrication technology)

4 Application example

The potential application of presented hybrid technologies in intraoral testing of biomaterials is enormous. In order to demonstrate a small fraction of this statement, a chip for specific use in intraoral environment was made (Fig. 9). The second hybrid fabrication technology with 3D printed middle layer in PLA was used. The sample of orthodontic NiTi archwire was inserted into the chip after fabrication and left to stand free in the microchannel. Artificial saliva with flow rate of 10 µL/min was inserted in the channel continuously for 24h.

No mechanical damage on the surface of the wire was evident (Fig. 10). The application of the microfluidic device, which does not alter the surface of the wire, is versatile and highly exploitable in medicine, dentistry as well as material sciences.

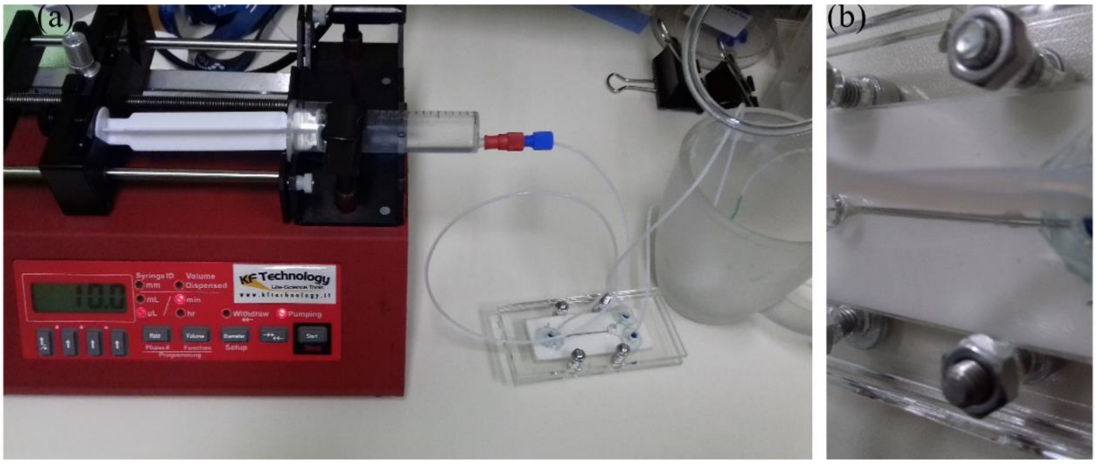

Fig. 9 Application example set-up: (a) syringe pump with connections, tubing and chip-holder with microfluidic chip and (b) closer view of the microchannel with inserted NiTi archwire in the middle

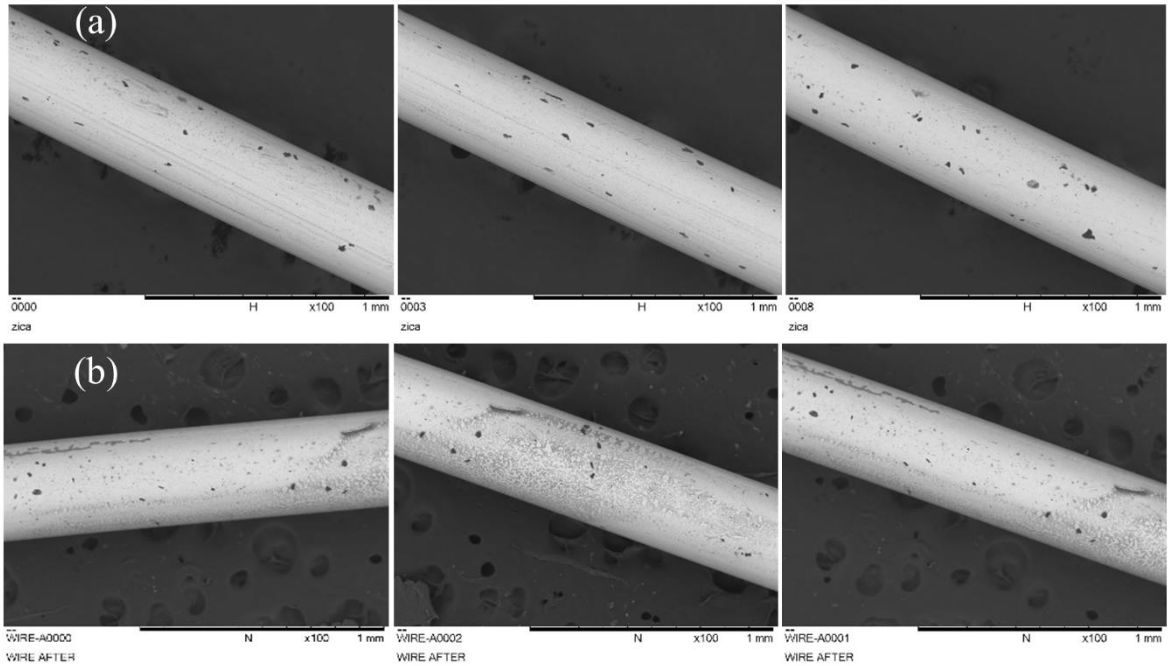

Fig. 10 SEM micrograph of archwires: (a) before and (b) after artificial saliva exposure

5 *Discussion*

Commonly used technologies for fabrication of microfluidic chips are time-consuming, require complex fabrication processes and sophisticated equipment. In order to overcome the mentioned disadvantages and simplify the fabrication procedure, two hybrid technologies are proposed. The proposed technologies combine 3D printing and xurography methods, allow rapid and robust manufacturing, use biocompatible materials and enable parallelization of the whole procedure for potential massive fabrication. In addition, the proposed chips are transparent, enabling optical detection of processes inside the microfluidic chip. The difference between two proposed technologies is in the bonding process between 3D printed and PVC layers – in the first hybrid fabrication technology the bonding was realized in hot lamination process and second hybrid fabrication technology combines 3M double adhesive tape and cold lamination process. The 3D printed layer slightly changes dimensions of the microchannels during the hot lamination process, thus the first technology, beside optimization of 3D printing and lamination parameters requires calculations of material expansion and prediction of channel dimensions after the lamination process. On the other hand, layer bonding in the cold lamination process with double-adhesive tape makes a stronger connection between layers compared with first technology and does not deform the channel dimensions. The tests of the chip durability made in both technologies showed that chips made with second hybrid fabrication technology have excellent durability performances and can handle up to 18 mL/min, while the maximum flow rate in microfluidic chips made in first hybrid fabrication technology was 3 mL/min. The proposed design and technologies have potential usage in making economical and simple tests with materials for potential biomedical applications due to similar conditions and behavior of fluid flow inside the body.

Manufacturing and testing microfluidic chips for controlled in vitro experiments and extraoral nickel salivary detection presents a promising field in the biomaterials assessment. The evaluation of exposure to nickel and other heavy metals could be substantially improved by the transfer of the contemporary diagnostics procedures onto portable, POC devices. Chips made in this way could be used for simulation of conditions present in the oral cavity and other body environments and for controlled and precise comparison between *in vivo* and *in vitro* tests. Microfluidic sensors for analyzing the presence of nickel, other heavy metals released from metallic appliances, and general salivary parameters in cases of heavy metals exposure, immunization and allergic reactions could be easily made. This includes the ranges of electrolytes (Na^+, K^+, Cl^-, F^-) analysis, general chemistries (pH, buffering capacity, urea, glucose, lactic acid) as well as saliva gases (pCO_2, pO_2).

The average value for unstimulated whole saliva flow in healthy person is between 0.1 and 0.3 mL/min. The normal range is very large and is in relationship with body position, exposure to light, previous stimulation, circadian rhythms, circannual rhythms, drugs, age, body weight, psychic effects, and functional stimulation (Tenovuo et al. 1994). In order to validate the possible application of the chips, we tested the range of artificial salivary flow that did not cause the leakage of the chips, in the range from 1 to 100 microliters per minute, flow rate that completely corresponds to physiological saliva flow. The rationale of using a NiTi orthodontic wire as a testing example is found in a fact that between 4.2 and 62% of persons with permanent teeth have a need for fixed orthodontic appliances (Caterini and Mezio 2017). In an everyday contemporary orthodontic practice, NiTi wires are exposed to harsh attacks. Since, the average treatment duration is 24 months, the fouling and degradation of wires in a combination with intraoral environmental and mechanical factors are present for a long time. The stress, strain, friction, abrasion, erosion and wear in combination with the corrosive environment (saliva, gastric acid, oral flora and byproducts) leads to degradation of the passive oxide layer and cause irreversible damage and consequently nickel release (Eliades and Athanasiou 2002). In addition, the variations in pH and temperatures, bacteria, medications, acid drinks, and chemoprophylactic agents, make tribocorrosion behavior of the NiTi wires uncertain. The measurements of corrosion and wear in a tribocorrosion system in vivo and the total amount of heavy metal ions in the saliva are challenging. The International Agency for Research on Cancer (IARC) classified nickel compounds as human carcinogens (IARC 1990). Nickel alone has been proved to cause toxic, carcinogenic, and immune-sensitizing effects (Das et al. 2010; Novelli et al. 2015). Release of nickel from medical devices may significantly contribute to the oral nickel intake up to 1 mg/day (Sabbioni and Hamdard 1985; Grandjean 1984; Cempel and Nikel 2006; FDA product Classification 2018). Nickel can induce genotoxicity, endocrine, neurogenic, gastrointestinal, cardiovascular, muscular, dermal, metabolic, immunological and carcinogenic effects. It is also potentially toxic based on the dose and duration of the exposure (Das et al. 2010). The implantation or intraoral use of nickel titanium biomedical devices carries a risk of pathologic conditions linked to nickel release in to the oral cavity or tissue. Up to 13% of the population is having various types of sensitivity to nickel, cobalt or chromium (Burkandt et al. 2011). Even though, numerous studies have been done in an attempt to confirm biocompatibility of NiTi alloys in the oral cavity, no convincing results have been obtained (Fercec et al. 2014; Williams 2015; Colic et al. 2016). The microfluidic chips could be an answer to that question. These hybrid technologies could find application in biomaterial testing (Caterini and Mezio 2017). They are very convenient for testing NiTi biomaterials used in oral cavity as a part of biomedical devices. Oral cavity is variable environment in regards to temperature changes, chemical composition and flow intensity. In order to demonstrate a small fraction of these claims, a chip for specific use in intraoral environment was made. We showed the behaviour of the NiTi wire sample in the chip with established flow rate of 1 microliter up to 2000 microliters per minute, suggesting possible efficient application in controlling the in vitro experiments mirroring the conditions of unstimulated or reduced saliva flow, together with simulation of hypersalivation condition or drooling. No mechanical damage on the surface of the wire was evident (Fig. 8). The application of the microfluidic device that does not alter the surface of the wire is versatile and highly exploitable in medicine, dentistry as well as material sciences. The specific salivary and other body fluids constituents that are considered as important biomarkers could be also studied *in vitro*, using developed microfluidic devices. The proposed design and technologies have a potential usage in making economical and simple tests with materials for potential biomedical application due to similar conditions and behaviour of fluid flow inside the body.

6 Conclusion

This paper studied an optimal combination of two cost-effective techniques for fabrication of sets of microfluidic chips. We integrated 3D rapid prototyping printing process with foils from xurography method and the compact multi-layered chips were created using thermal lamination. The effects of different types of chosen materials and manufacturing parameters on microfluidic chips characteristics were comprehensively analyzed. Morphological and structures performances of fabricated Y-shaped micromixers were examined using SEM and optical profiler. Variation in chips dimensions after manufacturing as well as maximal flow rate were also investigated. The microfluidic chips were tested on application example important for intraoral dentistry. Further research in this field will be directed towards combining new biocompatible, transparent and mechanically flexible materials such as Flexdym.

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