Timing and Controlling Dissolution of Cell Repellent Poly(Vinyl Alcohol) Thin Films for Patterned Cell Micro-Arrays

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Abstract

Recently, a novel, high-throughput, patterned cell micro-array has been developed using polydopamine and poly(vinyl alcohol) (PVA) as cell adhesive and cell repellent surfaces, respectively. The step converting the cell repellent surface to an adhesive one was shown to be toxic, so this project set out to investigate whether the cell repellent PVA surface could be dissolved instead through timed or controlled dissolution. PVA film solubility was tested in water and in cell compatible media. Various methods for stabilizing PVA films were investigated, hoping that they would show desirable dissolution profiles. The cell repellent properties of prepared films were assessed using HeLa cells. During solubility tests, a flaw was discovered and corrected in the previously established dissolution investigation method using wafers as substrates. None of the attempted preparation methods resulted in PVA films that had ideal dissolution profiles. At best, films would either dissolve completely or stabilize after only a few minutes. No method for reversing the PVA-film stabilization succeeded either. Thus new preparation and stabilization methods for PVA films should be investigated.
Sammendrag


Résumé

Récemment, une matrice à motifs cellulaires utilisant de la polydopamine et du poly(alcool de vinyl) (PVA) en tant que surface respectivement cytoadhérente et cytorépulsive a été développée. L’étape transformant la surface cytorépulsive en surface adhérente s’est avérée être toxique. Le but de ce projet fut donc d’examiner si la surface PVA cytorépulsive pouvait être dissoute par une dissolution chronométrée ou contrôlée non toxique. La solubilité des films PVA fut testée dans l’eau ainsi que dans des milieux favorables aux cellules. Différentes méthodes pour stabiliser les films PVA furent tentées espérant que ces films allait pouvoient se révéler avoir des pouvoirs de dissolution adéquates. Leurs capacités cytorépulsives furent évaluées à l’aide des cellules HeLa. Pendant les tests, un défaut fut découvert et corrigé par rapport à la méthode du test de dissolution précédemment établie. Aucune des méthodes de préparation tentées n’ont abouti sur des films PVA à profil de dissolution idéal. Au mieux, les films furent entièrement dissous ou stabilisés au bout de quelques minutes seulement. Aucune méthode inversant la stabilisation de films PVA n’a réussi. Ainsi, de nouvelles méthodes de recherche pour préparer et stabiliser les films PVA vont devoir être élaborées.
Preface

The work presented in this report is a contribution to the patterned cell micro-array project started by prof. Paweł Sikorski and PhD-candidate Kai S. Beckwith at the Bionanotechnology Lab at Dept. of Physics, NTNU. This report is the master’s thesis concluding Frédéric Ménard Lindboe’s master of technology degree in nanotechnology, specialization in bionanotechnology, study program at NTNU, completed in the spring semester of 2014. The work was done in NTNU NanoLab, in Biophysics Bionanotechnology lab and in Biophysics cell lab. This thesis is an extension of Frédéric Ménard Lindboe’s project work *Timed dissolution of poly(vinyl alcohol) and establishment of polymer film dissolution test for high-throughput patterned cell micro-array* [1] from the fall semester of 2013. For this reason some parts of the introduction, theory and methods chapters from the project report have been reused in this report. Some of the experiments done in the project work were repeated and an presented in the beginning of the results section as an introduction to and a reference for the following work.
## Contents

Abstract (English) .......................................................... iii
Sammendrag/Résumé (norsk/français) .................................... iv
Preface ........................................................................ v
Table of Contents ........................................................... viii
List of Tables .................................................................... ix
List of Figures .................................................................... xiii

1 Introduction and motivation ................................................. 1

2 Theory ........................................................................... 7
  2.1 Polymers ...................................................................... 7
  2.2 Hydrogen bonds .......................................................... 9
  2.3 Poly(vinyl alcohol) (PVA) ............................................. 9
  2.4 Cell adhesion ............................................................. 13
  2.5 Cell culturing ............................................................. 14
  2.6 Microcontact printing of polydopamine ......................... 15
  2.7 Photolithography ....................................................... 15
  2.8 Spin coating .............................................................. 17
  2.9 Topography and thickness measurement instruments ....... 18
    2.9.1 Reflectometer and interference patterns ................. 18
    2.9.2 Profilometer ....................................................... 19
  2.10 Light microscopy ...................................................... 19
    2.10.1 Köhler Illumination ........................................... 21
    2.10.2 Bright Field Microscopy ..................................... 21
    2.10.3 Phase Contrast Microscopy ................................ 21
    2.10.4 Differential interference contrast microscopy ........ 23
2.11 Conceptual descriptions of timed and controlled dissolution ............. 24
2.12 Borate and sodium sulfate stabilization of PVA .......................... 25
  2.12.1 Borate stabilization of PVA ...................................... 25
  2.12.2 Sodium sulfate stabilization of PVA ............................. 28

3 Methods ............................................. 29
  3.1 PVA thin film solubility testing ............................................. 29
    3.1.1 Determination of dissolution properties ............................. 29
    3.1.2 Solubility test experimental set up ................................. 33
    3.1.3 PVA thin film sample on wafer preparation .......................... 35
    3.1.4 Stabilizing soluble PVA thin films through exposure of various solutions ................................................................. 37
  3.2 Cell work .......................................... 38
    3.2.1 Cell culturing ...................................................... 38
    3.2.2 Set up for cell experiment .......................................... 39
  3.3 Patterning of PVA films ................................................. 40
    3.3.1 Microcontact printing ................................................ 40
    3.3.2 Photolithography ..................................................... 40
  3.4 Other chemicals used .................................................... 41

4 Results .............................................. 43
  4.1 Reference solubility tests ................................................ 43
  4.2 Controlled dissolution of water soluble low-PVA films ...................... 45
    4.2.1 Comparing dissolution properties of low-PVA thin films in cell medium, PBS and ethanol ..................................................... 45
    4.2.2 Cell repellence tests on low-PVA .................................... 51
    4.2.3 Solubility tests of low-PVA films of different concentrations ..... 53
    4.2.4 Low-PVA thin film patterning with photolithography .................. 55
    4.2.5 Summary of controlling the dissolution of low-PVA films with cell medium and PBS ................................................................. 56
  4.3 Stabilizing readily soluble PVA thin films ................................ 59
    4.3.1 Stabilizing PVA films in borate solutions ........................... 59
    4.3.2 Stabilizing PVA films in sodium sulfate solution .................... 68
    4.3.3 Stabilizing PVA films in mixed borate and sodium sulfate solutions ....................................................................................... 72
  4.4 Destabilizing insoluble PVA thin films with poly(acrylic acid) ............. 74

5 Discussion ............................................ 77
  5.1 Dissolution investigation method review ..................................... 77
  5.2 Controlled vs. timed dissolution .......................................... 78
  5.3 Thin films vs. bulk gels ................................................... 79
  5.4 Possibilities for using sodium sulfate to stabilize PVA films ............. 80
  5.5 Future prospects ......................................................... 80

6 Conclusion ........................................... 83

7 Appendix .............................................. 85
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Guiding values of geometrical parameters of strong, moderate, and weak hydrogen bonds</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>Properties of PVA</td>
<td>11</td>
</tr>
<tr>
<td>2.3</td>
<td>Properties of sodium sulfate</td>
<td>28</td>
</tr>
<tr>
<td>3.1</td>
<td>List of available PVA types with molecular weight and degree of hydrolysis</td>
<td>36</td>
</tr>
<tr>
<td>3.2</td>
<td>Chemicals used with illustrations and properties</td>
<td>41</td>
</tr>
<tr>
<td>4.1</td>
<td>Solubility tests of low-PVA in cell medium</td>
<td>47</td>
</tr>
<tr>
<td>4.2</td>
<td>Solubility tests of high-PVA in cell medium</td>
<td>47</td>
</tr>
<tr>
<td>4.3</td>
<td>Solubility tests of low-PVA in DMEM</td>
<td>48</td>
</tr>
<tr>
<td>4.4</td>
<td>Solubility tests of low-PVA in PBS</td>
<td>48</td>
</tr>
<tr>
<td>4.5</td>
<td>Solubility test of saturated borate induced PVA-hydrogels in water and glucose</td>
<td>65</td>
</tr>
</tbody>
</table>
List of Figures

1.1 Example confocal microscope images of cell arrays .......................... 4
1.2 Illustration of cell array fabrication and usage .................................. 4
1.3 Ideal profiles of timed and controlled dissolution of PVA films .......... 5

2.1 Illustration of a hydrogen bond between water molecules with geometrical parameters .................................................. 10
2.2 Chemical structure of PVA monomer .................................................. 11
2.3 Schematic representation of the crystalline region and the amorphous region of PVA .................................................. 12
2.4 Synthesis of poly(vinyl alcohol) from poly(vinyl acetate) ................. 12
2.5 Structure of the cell adhesion protein integrin .................................. 13
2.6 Example of cell growth curve .......................................................... 14
2.7 Illustration of microcontact printing .................................................. 16
2.8 Polymerization of dopamine to polydopamine .................................. 16
2.9 Illustration of photolithography principles with both positive and negative photoresists .................................................. 17
2.10 Spin coating ....................................................................................... 18
2.11 Reflectometer theory ........................................................................ 20
2.12 Illustration of Köhler illumination principles ....................................... 21
2.13 The principles of phase contrast microscopy ....................................... 22
2.14 The principles of differential interference contrast microscopy ........ 24
2.15 Cui et al.’s model for reversible PVA stabilization of borate cross-linking . 26
2.16 Manna et al.’s model for reversible PVA stabilization of borate cross-linking 27
2.17 Structure of sodium sulfate ............................................................. 28

3.1 PVA films on Si-wafer substrate with interference patterns with different colors for different thicknesses $d$ ........................................ 30
3.2 Reflectometer instrument and software ............................................... 31
3.3 Scratches on sample to be inspected in profilometer ............................ 31
3.4 Example of profilometer plots of PVA film samples on Si-wafer .......... 32
3.5 Wafer sample kept in solution with custom made device for quick and easy lowering .................................................. 34
3.6 Water bath preparation, dissolution testing and photographing of PVA films on Si-wafer ........................................... 35
3.7 PVA thin film sample on wafer with PVA free corner .................. 37
3.8 Example images of adhered, non-adhered and dividing cells .......... 40

4.1 Reference solubility tests in water for the films baked low-PVA, baked low2-PVA, unbaked high-PVA and baked high-PVA .................... 44
4.2 DIC image of tweezer scratches of low-PVA film samples incubated in PBS .......................................................... 49
4.3 Solubility tests of low-PVA in cell medium followed by PBS ........ 49
4.4 DIC image of tweezer scratches of low-PVA film samples after incubation in cell medium followed by incubation in PBS .................. 49
4.5 Solubility testing of low-PVA films in ethanol .......................... 50
4.6 Solubility testing of ethanol rinsed low-PVA films in cell medium followed by PBS treatment ........................................... 50
4.7 Cell repellence test of low-PVA thin films ............................... 52
4.8 Cell repellence test of polydopamine microcontact printed low-PVA thin films .......................................................... 54
4.9 Solubility test of low-PVA films made from solutions of different weight percentages in cell medium ................................. 55
4.10 Blank wafer incubated in cell medium for 20 s .......................... 55
4.11 Photolithography patterned low-PVA films ............................... 56
4.12 Profilometer scans of photolithography patterned squares on low-PVA films ......................................................... 58
4.13 Solubility test of baked low-PVA films stabilized in saturated borate ........................ 60
4.14 Solubility test of baked low2-PVA films stabilized in saturated borate ................... 60
4.15 Solubility test of unbaked high-PVA film stabilized in saturated borate .................. 60
4.16 Solubility test of baked high-PVA films stabilized in saturated borate .................. 61
4.17 Long solubility test of baked mixed PVA films stabilized in saturated borate .................. 62
4.18 Short solubility test of baked mixed PVA films stabilized in saturated borate .................. 62
4.19 Comparing long term solubility of baked borate stabilized and baked non-borate stabilized mixed PVA films ................................. 63
4.20 Solubility test of baked low-PVA films stabilized in low concentration borate ............... 64
4.21 Solubility test of unbaked high-PVA films stabilized in low concentration borate ........................ 65
4.22 Solubility test of unbaked high-PVA films stabilized in 50 mM borate at pH 10.0 ................................................................ 65
4.23 Low concentration borate induced high-PVA hydrogel formation .......................................................... 66
4.24 Saturated borate induced hydrogels made from both high-PVA and low-PVA ............... 66
4.25 Cell adherence test of aminophenylboronic acid coated high-PVA films ........................ 67
4.26 Verification of stability of unbaked high-PVA in sodium sulfate solution ........................ 68
4.27 Solubility test of unbaked high-PVA stabilized in sodium sulfate for various lengths of time .................................................. 70
4.28 Solubility test of baked and unbaked low-PVA stabilized in sodium sulfate ............... 71
4.29 Cell repellence test of sodium sulfate stabilized, polydopamine patterned, unbaked high-PVA films ................................. 71
4.30 Solubility tests of unbaked high-PVA films stabilized in a mixed solution of 0.5 M sodium sulfate and 5 mM borate at pH 8.8 ..................................... 72
4.31 Solubility tests of unbaked high-PVA thin films stabilized in a mixed solution of 0.5 M sodium sulfate and 5 mM borate at pH 10.2 .................... 73
4.32 Solubility tests of unbaked high-PVA films stabilized in a mixed solution of 0.5 M sodium sulfate and 50 mM borate at pH 9.9 .................. 73
4.33 Demonstration of water solubility of PAA-films ............................ 75
4.34 Solubility test of mixed high-PVA:PAA films .............................. 75
4.35 Solubility test of pH increased mixed high-PVA:PAA films ............ 76
4.36 Solubility test of pH increased mixed low-PVA:PAA films ............. 76

5.1 Conceptual illustrations of timed and controlled dissolution of PVA ............................. 79

7.1 Cell repellence test of low-PVA thin films incubated directly in cell medium with HeLa cells ............................................................. 86
7.2 Cell repellence test of low-PVA thin films incubated once in cell medium which was removed before cell seeding with new cell medium ......... 87
7.3 Cell repellence test of PBS incubated low-PVA films before cell seeding in cell medium ................................................................. 88
7.4 Cell repellence test of low-PVA films incubated in cell medium, then PBS before cell seeding in cell medium ........................................... 89
Chapter 1

Introduction and motivation

In modern society, many people take their health for granted. But overall good quality of life and long life expectancies have not always been the reality. Over the past century, proper medication has become readily available if you should get sick, and preventive vaccination has become the norm and has eradicated previously pandemic diseases. This has become possible through the accumulated understanding of cell biology [2]. Cells are the basic units of life. Understanding how cells function and how they respond to different stimuli is essential for knowing what do to if something should go wrong (if you get sick), or understanding how to prevent something from going wrong. Cell studies have gone through major improvements over the past century and have resulted in powerful applications. The understanding of fundamental cell biology and of common diseases such as cancer, and the drug development in medicine have all greatly benefited from cell studies. In multicellular organisms like humans, cells live and interact together. The most relevant way of learning about cell behavior would then be through in vivo studies. These, however, are generally unpractical, very resource intensive, quite time consuming to perform and in some cases even also unethical. Therefore, in vitro studies have been preferred for the study of cells [3]. However, the tools for in vitro cell studies remain quite simple. Traditionally, to study how cells respond to various stimuli, the cells are placed in a culture dish and stimulated (for example by being exposed to different proteins, different temperatures, to mechanical stress) [4]. The results are of often measured by investigating the response (e.g. protein expression or RNA concentration) of a group of cells, for example in one well or culture dish. This often results in averaging the cells’ response and would not detect different groups of cells responding differently. For studying morphology, a scientist would usually look around the dish for the cells behaving in the most desired manner, supporting the purpose of the experiment, thereby leaving a non-negligible fraction of the cells in the experiment out of the data. The different cell behaviors in these experiments could be due to hidden parameters influencing the behavior of each individual cell. These parameters could be cell morphology or the number of surrounding neighbor cells which a single cell can interact with. Therefore, there is a need to organize cells in a manner that allows for the mentioned parameters to be controlled in order to increase the fraction of
cells behaving similarly and thereby increase the throughput of data in cell experiments.

Cell arrays have been developed as techniques for patterning the substrate on which cells grow [5]. Designing the patterns for surfaces where cells are able and unable to grow will reduce freedom of the individual cells to migrate and tune the number of neighboring cells they can interact with. Patterning can also be done at single cell length scales to isolate cells from each other and shape the cell morphology after the patterns [6]. Cell arrays would therefore allow for cell studies being conducted with increased throughput. The patterning of cell arrays is a technique on the rise and has several interesting applications. Cell arrays have been used to study the relation between cell morphology and function [7], cell migration [8], and stem cell biology behavior [9]. Furthermore, co-cultures can be studied in cell arrays. In these, two or more different cell types can be spatially organized. Applications can be to maximize the contact interface between the cell types or to more precisely mimic real tissue structure in vitro. The understanding of the interaction between different cell types is crucial for increasing the knowledge about how cells behave in their natural environment. Thus it could lead to knowledge about diseases which again will push the development of novel cures. The key to making patterned cell arrays is to control where cells can and cannot attach themselves. Cells attach to surfaces because their membrane bound proteins are able to adhere to them. Compounds such as PEG have been reported to serve as cell repellent surfaces [10], and for the cell adhesive surface alkanethiols have been used [11]. In order to pattern the arrays, techniques such as photolithography [12], stencil patterning [13], and soft lithography [14] have been employed. Examples of patterned cell arrays are shown in figure 1.1.

Recently, a novel patterned cell array system was developed at NTNU using simple chemistry, simple methods and readily available and relatively cheap chemicals. The goal is to create a high-precision and high-throughput cell study platform that can readily be fabricated and used by a wide range of cell biology labs without the need for expensive equipment or custom materials [15]. The compounds chosen for cell adhesion and anti-fouling in this project are the off-the-shelf chemicals polydopamine (PD) and poly(vinyl-alcohol) (PVA), respectively. The advantage in using these polymers is that they are almost universally protein adhesive [16] and repellent [17], respectively. This means that almost any adherent cell type can be studied using this cell array, making the potential use of it very wide (red blood cells for instance grow in suspension [18] and are not adherent). This cell array was made through microcontact printing of PD patterns on a PVA substrate (see figure 1.2). A cell population was then deposited onto a patterned surface and the cells would only grow on the cell adhesive PD surfaces. For single cell migration studies or for co-culture studies, the next step involved in converting the cell repellent PVA surface to a cell adhesive one. The strategy opted in the cell array in question is to deposit dopamine onto the cell array and make it polymerize and form a cell adhesive PD layer on the previously cell repellent area. The challenge, however, is that this procedure is somewhat toxic (it generates reactive oxygen species) and could compromise the future cell studies.

The motivation behind the work presented in this report is the desire to circumvent this problem. Instead of creating a new cell adhesive PD layer on top of the cell repellent PVA layer, it might be possible to remove the cell repellent layer, exposing a new cell adhesive layer. The idea is to simply dissolve the cell repellent PVA layer after the deposited cells have attached to the cell adhesive surface. In practice, there are two possibilities for
Chapter 1. Introduction and motivation

achieving this. The first one is that the PVA layer would need to dissolve slowly, while the
cells attach to the PD surface, so that the PVA layer does not dissolve completely before
cells have adhered to the PD patterns after a few hours (timed dissolution). The second one
is that the PVA layer is stable, but dissolves quickly when some compound is added to the
cell array that destabilizes the PVA film after the cells have attached to the PD patterned
areas (controlled dissolution). For timed dissolution an, ideal dissolution profile would be
that is dissolves at a linear rate (see figure 1.3a), making it easy to predict the time needed
for complete dissolution to occur. For controlled dissolution, it would be ideal that the
added PVA dissolution would occur quickly and begin immediately after the introduction
of PVA destabilizing compound (see figure 1.3b). This must all happen in 37°C incubation
so that human cell lines can be studied. The work of this report sought to find out whether
it was possible to prepare the cell repellent PVA layer in such a way that these kinds of
dissolution profiles could occur. A method for investigating the dissolution profile of PVA
films was previously developed [1] and was adopted in this project. We used PVA types
of different degrees of hydrolysis and attempted to stabilizing the prepared PVA films by
baking, borate induced cross-linking and sodium sulfate "salting out". We further tested
the cell repellent properties of the developed PVA film types using HeLa cells.
Chapter 1. Introduction and motivation

Figure 1.1: Example confocal microscope images of cell arrays [15]. Scale bar: 200 mm. Cell labeling: (a) actin filament (b) green: calcein-AM, red: CellTracker.

Figure 1.2: Illustration of cell array fabrication process and usage [15]. The process involves micro-contact printing of polydopamine onto PVA films, seeding and growing of cells onto polydopamine patterns before covering the cell repellent PVA surfaces with dopamine and polymerizing it into polydopamine so new cell types can grow on there in order to make patterned a co-culture.
Figure 1.3: Ideal profiles of timed and controlled dissolution of PVA films. Red lines: PVA film thickness, green lines: cell adherent properties of PVA coated surface. The concepts of both timed and controlled dissolution are explained in the text.
Chapter 2

Theory

2.1 Polymers

Polymers are macromolecules consisting of chains of covalently linked repeated units (monomers) [19]. Polymers range from synthetic polymers like plastics to natural polymers like DNA or RNA. Due to their long chain like structure they have special properties with respect to toughness, viscosity and elasticity. Polymers can be charged, uncharged as well as ampholytic (capable of acting as both base and acid) [20]. Polymers are often described with the parameters described below.

General properties of polymers

Polymer properties depend on several parameters which are explained below. As poly(vinyl alcohol) (PVA) is a polymer which will form a solid thin film, certain polymer film properties are explained as well. Some specifications regarding PVA are included.

Molecular mass/weight \( M_n = \sum_n M_0 \). Total mass of a polymer where \( M_0 \) is the mass of the monomer and \( n \) is the number of monomers in the polymer. \( M_n \) is practically used to designate the mean molecular mass of the polymers in a large population. Molecular masses of polymers are usually given in the units of kiloDaltons (kDa), where the Dalton equals to the atomic mass unit \( u \) and 1 Da = 1 u = 1.66053886(28) \( \cdot 10^{-27} \) kg [21]. 1 Da is the equivalent of 1 g mol\(^{-1}\).

Degree of polymerization The number of monomeric units in a macromolecule or polymer or oligomer molecule [22]. For a homopolymer, a polymer with only one single type of monomer such as PVA, it is defined as \( DP_n = \frac{\overline{M_n}}{M_0} \), where \( \overline{M_n} \) is the average molecular mass of the polymer and \( M_0 \) is the mass of its monomer.

Disperity \( D = \frac{\overline{M_w}}{\overline{M_n}} \). Measure of the heterogeneity of molecule or particle sizes in a mixture. It gives an indication of the distribution of individual molecular masses in a batch of polymers. The disperity is defined the ratio between the weight the weight
average molecular weight $\overline{M}_w = \sum_i n_i M_i$ and the number average molecular weight $\overline{M}_n = \sum_i n_i M_i / \sum_i n_i$, where $M_i$ is the molecular mass of a polymer and $n_i$ is the number of polymers with molecular mass $M_i$. The number average molecular weight is the total mass of the population $\sum_i n_i M_i$ divided by the total number of polymers. The weight average molecular weight is the sum of the molecular mass $M_i$ multiplied by the weight fraction $n_i M_i$ (the weight of all polymers with a particular molecular mass divided by the weight of the total population). The dispersity is greater or equal to 1, it approaches 1 as the polymer molecular mass distribution gets more uniform. The term dispersity replaces the misleading term "polydispersity index" [23].

**Persistence length** The persistence length is a measure of the stiffness of a polymer. Essentially it is the length scale at which a polymer is roughly straight [24]. When the polymer chain is longer than the persistence length, thermal energy is able to bend the chain. If one looks at two points of a polymer chain and compare the angle between their tangents, the correlation will drop exponentially from 1 for two infinitely close points to 0 for two very distant points. The persistence length is a measure of this decay and is the length at which this correlation is lost [25]. The longer the persistence length is, the stiffer is the polymer.

**Properties of solid polymer films**

**Degree of crystallinity** The fractional amount of crystallinity in the polymer sample [19]. It is expressed either as a mass fraction ($w_c$) or as a volume fraction ($\phi_c$). The mass fraction and the volume fraction are related by $w_c = \rho_c \phi_c$ where $\rho_c$ and $\rho_p$ are the densities of the crystalline fraction and of the entire sample, respectively. For PVA the crystallization is determined by the formation of hydrogen bonds between the alcohol groups of the polymer chains. Its value usually ranges between 30% and 60% [26].

**Lamellar thickness** The thickness of the two dimensional layer of crystallite resulting from the folding of polymer chains. In PVA the chains are held together by hydrogen bonds [27, 28].

**Properties of solid PVA films**

**Degree of hydrolysis** For PVA it is the fraction of ester groups (-COOCH$_3$) converted to hydroxyl groups (-OH) when poly(vinyl alcohol) is synthesized from poly(vinyl acetate) through hydrolysis. A description of PVA synthesis is offered in section 2.3. Ester groups do not form hydrogen bonds as easily as hydroxyl groups and therefore contribute less to the crystallization of PVA.

**Glass transition temperature** $T_{gt} = 85$°C for PVA [29], temperature above which the polymer has got enough thermal energy to rearrange its chains so they can create hydrogen bonds among themselves.
Chapter 2. Theory

2.2 Hydrogen bonds

Hydrogen bonds between the -OH groups of PVA are essential for the stabilization of PVA films and therefore also for its solubility [30]. Since the dissolution of PVA is an essential part of the work in this report, hydrogen bond will be described below.

Hydrogen bonds are commonly known as an interaction between a hydrogen atom of one molecule with an oxygen atom of another [31]. They are the reason for the unique properties of water as a polar solvent. Hydrogen bonds are also important as a stabilizing agent between macromolecules in solution. The hydrogen bond is a weak interaction. The energy cost of breaking a macromolecule-macromolecule hydrogen bonds and making a macromolecule-water molecule hydrogen bonds instead is only in the order of a few $k_B T_r$, where $k_B$ is Boltzmann’s constant and $T_r$ is the room temperature [31]. Its stabilizing properties arise from the large number of hydrogens bonds made by the macromolecules. Generally, hydrogen bonds (H-bonds) are defined as a non-covalent, electrostatic association between an electronegative atom A and a hydrogen atom H covalently linked to a second, relatively electronegative atom X [19]. The electronegative atom A can be referred to as the hydrogen bond acceptor and the hydrogen-electronegative atom pair H-X is referred to as the hydrogen bond donor [32]. A hydrogen bond can be illustrated as X–H···A, where the dash (–) symbolizes a covalent bond and the dots (···) symbolize the hydrogen bonding. Hydrogen bonds can be intramolecular or intermolecular. The electronegative atoms are often, but not exclusively, from the first row of the Periodic Table, such as oxygen (O), fluorine (F) or nitrogen (N). In 2011, IUPAC published an update definition for hydrogen bonds due to recent theoretical and experimental advances [32]:

The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation.

Hydrogen bonds are short range forces. Although there is a continuum of hydrogen bond strengths it can be useful to classify them in categorizes: strong, moderate and weak [33]. Their geometrical parameters are summarized in table 2.1 and illustrated in figure 2.1 where $d$ is the distance H···A, $D$ is the distance between the two electronegative atoms X···A of the hydrogen bond, and $\theta$ is the angle between X–H and H···A.

2.3 Poly(vinyl alcohol) (PVA)

In the cell array project, poly(vinyl alcohol) is the cell repellent layer. The work presented in this report involves its dissolution. Therefore understanding the fundamentals about poly(vinyl alcohol) is crucial.

Poly(vinyl alcohol), shortened PVA, is a water soluble polymer [37] whose chemical structure is illustrated in figure 2.2. PVA can be in three different states. The first one is a crystallite state where PVA chains are strongly bound to each other through numerous hydrogen bonds. The chains fold up and down in a snake-like manner creating a two dimensional layer with a certain lamellar thickness $L$. The second state is an amorphous
2.3 Poly(vinyl alcohol) (PVA)

Figure 2.1: Illustration of a hydrogen bond between water molecules with geometrical parameters [33]

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<thead>
<tr>
<th></th>
<th>Strong</th>
<th>Moderate</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$, H···A [Å]</td>
<td>1.2–1.5</td>
<td>1.5–2.2</td>
<td>&lt;2.2</td>
</tr>
<tr>
<td>lengthening of X-H [Å]</td>
<td>0.08–25</td>
<td>0.02–0.08</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>$D$, X···A [Å]</td>
<td>2.2–2.5</td>
<td>2.5–3.2</td>
<td>&gt;3.2</td>
</tr>
<tr>
<td>X–H versus H···A</td>
<td>X–H≈H···A</td>
<td>X–H&lt;H···A</td>
<td>X–H&gt;H···A</td>
</tr>
<tr>
<td>interaction type</td>
<td>strongly covalent</td>
<td>mostly electrostatic</td>
<td>electrostat./dispers.</td>
</tr>
<tr>
<td>directionality</td>
<td>strong</td>
<td>moderate</td>
<td>weak</td>
</tr>
<tr>
<td>bond angles $\theta$ [°]</td>
<td>170–180</td>
<td>&gt;130</td>
<td>&gt;90</td>
</tr>
<tr>
<td>bond energy $E$ [kcal·mol$^{-1}$]</td>
<td>15–40</td>
<td>4–15</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

Table 2.1: Guiding values of geometrical parameters of strong, moderate, and weak hydrogen bonds [34]

hydrogel state, where the chains are mostly bound to water molecules but also, to a limited extent, to neighboring chains. The third and last state is a completely dissolved state where each polymer chain only interacts with water and nothing else. Dissolution does not involve the breaking of covalent bonds. It is important to note that different regions of the same chain can be in different states. The crystallite state and the amorphous state are illustrated in figure 2.3.

Synthesis of PVA

The monomer vinyl alcohol (CH$_2$=CHOH) does not exist, as its tautomeric equilibrium lies on the acetaldehyde side, the double bond and the hydrogen atom of the hydroxyl group (-OH) in vinyl alcohol molecule will under ambient conditions spontaneously reorganize and yield acetaldehyde (CH$_3$CH=OH) [38]. Therefore the synthesis of PVA is made indirectly through other polymers. The most important manufacturing process is polymerization of vinyl esters or ethers followed by saponification or transesterification. The most common starting material is vinyl acetate (CH$_3$COOCH=CH$_2$). The polymerization of poly(vinyl acetate) (PVAc, [CH$_3$COOCHCH$_2$]$_n$) is done in methanol (CH$_3$OH). The factors determining the molec-
Chapter 2. Theory

2.3 Poly(vinyl alcohol) (PVA)

![Chemical structure of PVA monomer](image)

**Figure 2.2:** Chemical structure of PVA monomer

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>([\text{CH}_2\text{CH(OH)}]_n)</td>
</tr>
<tr>
<td>Molecular weight of monomer (M_0) [35]</td>
<td>44.053 Da</td>
</tr>
<tr>
<td>Refractive index of PVA thin film (n) [36]</td>
<td>1.506-1.531</td>
</tr>
<tr>
<td>Glass transition temperature (T_{gt}) [29]</td>
<td>85°C</td>
</tr>
</tbody>
</table>

**Table 2.2:** Properties of PVA

Molecular mass (and chain length) of PVAc and of the subsequent PVA are polymerization temperature, vinyl acetate-methanol ratio and polymerization conversion. Lowering these factors increases molecular mass. PVAc then undergoes hydrolysis when reacting with methanol catalyzed by sodium methoxide (\(\text{CH}_3\text{ONa}\)) or sodium hydroxide (\(\text{NaOH}\)) and yields poly(vinyl alcohol) (see figure 2.4) and methyl acetate (\(\text{CH}_3\text{COOCH}_3\)) as a by-product. By varying the catalyst concentration, reaction temperature and the reaction time the fraction of conversion of the ester group (-\(\text{OCOCH}_3\)) to the hydroxyl group (-\(\text{OH}\)) can be tuned. For PVA, this fraction is called the degree of hydrolysis.

**PVA properties**

The properties of PVA depend on several general parameters important for many polymers which are described in section 2.1 on page 7. The more specific and practical properties of PVA are described here.

PVA has excellent film forming and surface adhesion properties [15, 17, 40]. Thin films of PVA can be made through techniques as dip coating or spin coating, the latter being described in section 2.8 on page 17. PVA has got high resistance to non-specific adsorption of proteins [17], making it an ideal substrate as a cell repellent surface in cell arrays [15]. PVA holds its cell repellent properties [41] from the permanent hydrophilic surface of the polymer [42]. When PVA crystallizes it can form a stable hydrogel [43] (three dimensional, cross-linked networks of water soluble polymers [44]). The PVA hydrogel is stabilized through hydrogen bonds. Hydrogen bonds are explained in detail in section 2.2 on page 9.

Hydrogels have gained interest in biomedical research as they have tunable properties such as elasticity, they can readily be functionalized [45], they have structural similarities to many extracellular matrix tissues and can be delivered in a minimally invasive manner [46]. PVA is one of these hydrogels and has many interesting pharmaceutical and biomedical applications [47]. PVA is neither toxic nor carcinogenic, it exhibits a high degree of swelling in water or biological fluids and is rubbery and elastic of nature. PVA is therefore capable of mimicking natural tissue and can be accepted into the body.

PVA is capable of making stable insoluble crystallite films after heat treatment. The literature reports annealing times of 15 to 90 minutes in temperatures of 90 to 120°C [27] for
making stable hydrogel films. As annealing time increases, the degree of crystallinity can reach up to 48% [48].

**Dissolution mechanism**

When PVA hydrogels dissolve the crystallite region first unfolds and becomes amorphous but not dissolved, before the amorphous region disentangles and finally dissolves. Both the unfolding and the disentanglement happen simultaneously in different parts of the polymer chain. So when the polymer is looked at as a whole or several polymers are looked at at the same time, its can be observed in three steps [27]:

1. Most of the PVA is crystallite so the greatest change will be the crystallite region of the polymer gradually unfolding to join the surrounding amorphous region. This results in a sudden drop of the degree of crystallinity.

2. As more of the polymer becomes amorphous, the amorphous region gradually disentangles while the unfolding of the crystallite region persists. This happens at very similar rates so that the degree of crystallinity is fairly constant.

3. The crystallite regions eventually all become unfolded and the amorphous region is in majority, so the observed degree of crystallinity drops to zero. Eventually all of the amorphous region disentangles and dissolves completely.
2.4 Cell adhesion

Most cells are anchorage dependent, they will not grow and proliferate unless they are attaching to some substrate. Traditional cell culturing substrates are hydrophilic and charged. Historically glass has been used due to its charged nature, but hydrophobic plastics treated with radiation, chemicals or ions have become normal to use for cell culture as many cell types prefer this substrate to glass [18].

Cell adhesion molecules are often transmembrane proteins [2], they span from the cytosol (the inside of the cell) out to the extracellular environment through the cell lipid membrane into which they are bound. Common cell-environment adhesion molecules in animal cells are integrins. They bind cells to the surrounding extracellular matrix (ECM) within organisms. Integrin is a heterodimer consisting of two non-covalently bound subunits $\alpha$ and $\beta$ [50]. Together, the extracellular portions of the integrin subunits interact to form a particular binding site (see figure 2.5). There are many types of subunits, one type of $\alpha$ subunit is free to bind to any other type of $\beta$ subunit, and vice-versa. Thus, there is a great number of different binding sites that can form making integrin a versatile cell adhesion protein. Integrins recognize and bind to certain glycoproteins in the ECM. The recognized glycoproteins often, but not exclusively, contain the RGD sequence containing the three amino acids arginine (abbreviated R or Arg), glycine (G or Gly) and aspartic acid (D or Asp) in this order [51].

Arginine and aspartic acid have got charged side chain (positive and negative, respectively) and glycine only has a hydrogen atom for a side chain. The RGD binding site of integrins is also capable of binding divalent cations and other charged residues [52]. Other substrate binding proteins are proteoglycans. These are involved in formation focal adhesions, where transmembrane adhesion proteins gather and form a local binding unit to the environment [50].

It is also possible to coat glass or plastic surfaces with functional domains of the ECM to improve cell adhesion in culture [53]. Some surfaces can actually start as cell repellent but later become cell adhesive because some cells actually secrete ECM proteins themselves onto the substrate. So an important parameter for cell adhesion on a surface can be the ability of ECM proteins to bind to the surface. Thus cell repellence is not only due to the inability of transmembrane adhesion proteins to bind to surfaces but the inability of ECM proteins of binding to them as well.

Figure 2.5: Structure of the cell adhesion protein integrin [49]
2.5 Cell culturing

When doing cell studies one uses cell lines [54]. A cell line is a culture of cells sharing the same genotype and phenotype. In cell studies, cell lines are used to prevent statistical variation due to differences in gene expression. An illustrative example of the growth of a cell culture is given in figure 2.6. After the initial seeding of a cell culture there is a lag phase where the cells attach to the culturing substrate and do not divide. Then comes a period of exponential growth, called the log phase. This phase continues until all the available substrate already is occupied by cells or the cell medium in which the cells grow is exhausted. Then the cell growth reduces and cell development reaches the plateau phase or the stationary phase. If nothing is done at this point, cells will eventually die and the culture reaches the death phase. To prevent cell death and to maintain cell lines cell splitting, or subculturing, is performed. This involves removal of the cell medium and dissociation of the cells with the substrate and with each other using trypsin. The cells are then diluted and reseeded onto a new substrate with fresh cell medium. It also normal to change the cell medium of cells in between splitting, during the log phase. When cell lines experience exponential growth, their time population number \( N(t) \) as a function of time \( t \) is given as follows:

\[
N(t) = N(0)e^{rt}
\]  

(2.1)

where \( N(0) \) is the initial population number at seeding, and \( r \) is the growth rate. For this project we used HeLa cells. HeLa cells are epithelial cervical cancer cells derived from patient Henrietta Lacks in 1951 [55]. It is an immortal cell line and the most commonly used human cell line today. The HeLa cell doubling time, how often cells undergo mitosis, is of approximately 23 hours [56].

Figure 2.6: Example of cell growth curve [57]. See text for explanation of the phases.
2.6 Microcontact printing of polydopamine

Microcontact printing is a soft lithography technique using micro-size featured stamps to pattern surfaces with chemical substances or with particles [58]. In this project microcontact printing is used to pattern cell adhesive surfaces onto cell repellent films. It is particularly valuable in the patterning of biological materials [59]. The microcontact printing procedure is illustrated in figure 2.7. After preparation of the stamp (master) it is coated with the substance to be printed (ink). The stamp is then pressed against a substrate to transfer the ink and pattern the substrate. The stamps are made from an elastomer, usually PDMS (polydimethylsiloxane) [60]. One advantage of this technique is that it can be used to pattern non-flat surfaces, contrary to other lithography techniques such as photolithography. One often encountered problem with microcontact printing can be unwanted contact of the stamp with the substrate due to "roof collapse" [61] (see figure 2.7b). This can happen when too much force is applied on the stamp during printing causing the grooves of the stamp to be pressed down and make contact with the surface depositing ink on unintended areas.

In the cell array project, polydopamine (PD) is the cell adhesive layer [15]. Polydopamine is a cell compatible material [62] and can be microcontact printed to form cell array patterns [63, 64]. Polydopamine is synthesized through oxidation of dopamine on a surface in mild alkaline solutions (see figure 2.8). The poly dopamine will self-assemble and form a thin film [65]. Dopamine, or 3,4-dihydroxyphenethylamine, is a derivative from the mussel adhesive protein DOPA (3,4-dihydroxyphenylalanine), secreted by marine mussels [66]. Although the detailed mechanisms of polydopamine’s protein adherence are unknown it involves cross-linking reactions leading to solidification of the secreted liquid adhesive protein [67].

2.7 Photolithography

Photolithography is a microfabrication technique using light to pattern thin films or substrate surfaces [68]. In this project we used photolithography to pattern PVA films. The patterning is done by first coating a sample with a UV-light sensitive photoresist. The photoresist is then UV-exposed through a chromium mask which covers parts of sample surface. Next, the resist is developed by removing either the UV-exposed resist or the non UV-exposed resist. Finally, the entire sample is etched. The remaining resist is protecting certain parts of the underlying film/substrate while the uncovered parts of the film/substrate are etched. An illustration of a photolithographic process is offered in figure 2.9. Photoresist are organic compounds, often polymers, that experiences a change in solubility in a developer solution upon UV-exposure [69]. There are two main kinds of photoresist: positive and negative, based on how the resist reacts to light exposure. Positive resists break down during UV-exposure and are made more soluble. The remaining photoresist pattern after development will therefore look like the mask (where the resist is equivalent to the chromium pattern), hence the name "positive". Negative resists, on the other hand, are cross-linked and hardened by UV-exposure. The developer solution then removes the non UV-exposed resist and the resulting resist pattern is a negative image of the chromium mask pattern.
2.7 Photolithography

Chapter 2. Theory

(a) Stamp coating and printing procedure

(b) Roof collapse

Figure 2.7: Illustration of microcontact printing [60, 61]. (a) The stamping procedure is described in the text. (b) Illustration of "roof collapse" and unwanted printing of surfaces if too much force (illustrated by the arrow) is applied to the PDMS stamp.

Figure 2.8: Polymerization of dopamine to polydopamine [63]
2.8 Spin coating

Spin coating is a widely used technique used in microfabrication. It is used for creating thin films from compounds in solution, in this project for the preparation of PVA thin films on glass slips or Si-wafers. A sample substrate is placed onto a chuck and is held on by a vacuum pump. After the substrate is covered with the solution of interest the sample is spun, usually around an axis going through its center, in order for the solution liquid to spread over the entire sample. Excess solution is cast off the sample due to centrifugal forces but a thin film will eventually remain as the viscosity quickly increases due to solvent evaporation and the thinning of the film (see figure 2.10a for illustration). The final film thickness will primarily depend on the spin coating velocity and the viscosity of the solution. The spin coat velocity \( \omega_{sc} \) increases with the centrifugal force \( F_c \) as:

\[
F_c = m \frac{v^2}{r} = mr \omega_{sc}^2,
\]

where \( m \) is the mass of the solute molecule, \( r \) is the distance of the molecule from the spin axis and \( v \) is the tangential speed of the molecule (\( \omega = \frac{v}{r} \)). Therefore higher spin coat velocities will be able to throw off more solution before it stabilizes and results in a thinner film. The viscosity increases with the polymer size, the concentration of the deposited solution or the evaporation of the solvent during spin coating. Increased viscosity results in thicker spin coated films. An example spin curve of films showing the relation between film thickness, spin velocity and viscosity is shown in figure 2.10b.
Edge beads are the accumulation of solute on the edge of the substrate after spin coating resulting in higher film thickness on the sample edges. This effect is due to increased surface stress at the borders [72].

![Film thickness as a function of spin coating time](image1)

![Example spin curve of ma-N 400 photoresist series](image2)

Figure 2.10: Spin coating

### 2.9 Topography and thickness measurement instruments

In this section the instruments used for surface topography measurement as well as the underlying principles for their functioning are described.

#### 2.9.1 Reflectometer and interference patterns

In order to calculate the thickness of a thin film on a reflective surface, a reflectometer measures the intensity of reflected light over a range of wavelengths. The reflectometer lamp exposes the sample with light a right angle and it will reflect back into a detector next to the lamp (see figure 2.11a for illustration). As incoming light hits the sample surface, some light waves will reflect back on the film-air interface (red line) and some will refract into the film and reflect on the film-substrate interface (blue line). In the illustration all of the light is of the same wavelength and is illustrated in different colors for clarity. For aiding in visualization in the following explanation, the light reflected on the film-air interface will be referred to as the red line and the light reflected on the film-substrate interface as the blue line. These two different reflected light waves will then interfere on the way back towards the detector, as they are aligned. The extra optical path length

---

^1Product of geometric path length and refractive index.
travelled of the blue line in the film of distance $2dn$, where $d$ is the film thickness and $n$ the refractive index of the film, will then determine the phase difference between the blue line and the red line. The phase difference will determine how they interfere and the intensity of the resulting wave. For maximum constructive interference to occur the extra travelled optical path length must be $\frac{m+1}{2} \frac{\lambda}{n}$ and for destructive interference the extra travelled path length must be $\frac{m}{n} \lambda$, where $m$ is an integer. For a given film thickness $d$, the reflectometer will measure the intensity of reflected light (interference between red and blue line) over a range of wavelengths around the visible spectrum. The intensity will vary with the wavelength as the phase difference between the red and blue line will depend on the wavelength. If we have two waves with amplitudes $A_1$ and $A_2$ the resulting intensity $I$ for a given wavelength $\lambda$ is:

$$I(\lambda) = A_1^2 + A_2^2 + 2A_1A_2 \cos(\Delta \phi) \quad (2.3)$$

$$\Delta \phi = \frac{2\pi l_{\text{opt}}}{\lambda} \quad (2.4)$$

$$l_{\text{opt}} = nl \quad (2.5)$$

where $l_{\text{opt}}$ is the optical path length, $l$ the geometrical path length, $n$ the refractive index and $\Delta \phi$ is the phase difference between the two waves. We have here used $\tau = 2\pi$ as a matter of principle [75]. A plot of equation 2.3 is shown in figure 2.11b.

The reflectometer software will then compare the measured intensity spectrum over the wavelength range (see figure 2.11c) with the ideal calculated spectrum for different film thicknesses. The measured film thickness $d$ is the thickness yielding the best fit between the measured intensity spectrum and the calculated spectrum. This match is measured as a goodness of fit from 0 to 1, where 0 is no correlation and 1 is perfect fit between measured and calculated intensity spectrum.

The resulting color of the interference pattern of the PVA film on the wafer that can be seen in regular light is a result of the different contributions of different colors of white light as the different colors reflected on sample will have different intensities.

### 2.9.2 Profilometer

In order to measure the topography of a surface a profilometer moves a stylus along a one dimensional path that records height differences and sends data to a computer. Since the measured height differences are relative to an arbitrary plane of reference within the instrument, leveling is often needed by aligning two points to make a reference for the zero height. This can be done in the instrument software.

### 2.10 Light microscopy

In this section the principles behind the microscopes used for investigation of PVA dissolution are explained.
2.10 Light microscopy

(a) Illustration of interfering waves for PVA films on Si-wafers. The red and blue lines are separate waves but of the same wavelength, the color difference is only for illustration purposes. The incoming and the reflected light are in fact overlapping but are separated for clarity.

(b) Interference intensity \( I \) as a function of \( \lambda \) calculated from film thickness of 20 \( \mu \)m (red), 2 \( \mu \)m (blue) and 400 nm (green) and \( n = 1.5 \) and equation 2.3.

(c) Example intensity/reflectance of the visible spectrum of a thin film measured in reflectometer [76]

Figure 2.11: Reflectometer theory
2.10.1 Köhler Illumination

The goal of Köhler illumination is to get the illumination of the sample as even as possible. The light source will never emit light with the same intensity from every point, especially if the light source uses a coiled filament. Even illumination achieved through assuring that the light coming from the lamp source is completely defocused when it reaches the sample plane. When looking into the microscope and the sample is in focus (research tends to get easier this way) the light source will also be defocused when it reaches the eye/camera. Köhler illumination is verified by focusing on the aperture diaphragm with the condenser lens because the sample plane and the aperture diaphragm are in each other’s image planes. Köhler illumination is illustrated in figure 2.12.

![Diagram of Köhler illumination](image.png)

Figure 2.12: Illustration of Köhler illumination principles. See the text for explanation.

2.10.2 Bright Field Microscopy

Bright field microscopy is a simple optical illumination technique. The sample is illuminated with white light. Light transmitted through the sample is light seen in the microscope. Contrast is achieved through absorbance or scattering of light in denser areas of the sample. The name bright field comes from the fact the light surrounding the sample is seen by the eye as a bright background light.

2.10.3 Phase Contrast Microscopy

Phase contrast microscopy is a useful technique for imaging transparent samples, like biological samples. It creates contrast from the phase change light experiences when traveling through a new material of different refractive index. This phase change is further increased in a phase plate so that the component of the transmitted light that has interacted with the sample (the diffracted or D-wave) is completely out of phase with the component of the
light that has not been phase delayed by the sample (the surround or S-wave). This is achieved with a phase plate that is placed out of focus for the D-wave. Illustrations are given in figure 2.13 and a quantitative description will follow.

The initial electromagnetic field $E_i$, the one between the light source and the sample, can be described by a simple sinus wave:

$$E_i = E_0 \sin(\omega t)$$

(2.6)

After interacting with sample, the field is phase shifted by $\phi$ where it has interacted with the sample. The phase modulated field $E_{PM}$ then becomes:

$$E_{PM} = E_0 \sin(\omega t + \phi)$$

(2.7)

where $\phi$ is a function in space.

Using a trigonometric identity and assuming that the phase shift $\phi$ is small ($\sin \phi \sim \phi$ and $\cos \phi \sim 1$) the electromagnetic field that has interacted with sample $E_{PM}$ becomes

$$E_{PM} = E_0 (\sin \omega t \cos \phi + \cos \omega t \sin \phi)$$

$$\sim E_0 (\sin \omega t + \phi \cos \omega t)$$

(2.8)

(2.9)

From equation 2.9 the phase modulated field can be interpreted as the sum of the S-wave, being the same as the field would have been without any sample, and the D-wave being the modified part of the field. Now one can also see that the S-wave and the D-wave are phase shifted by $\frac{\lambda}{4}$ in space ($\lambda$ being the wavelength of the light), or by $\frac{\pi}{4}$ radians. By the means of the phase plate the S-wave, which is in focus at the phase plate plane, is delayed by an additional $\frac{\lambda}{4}$ in space, or $\frac{\pi}{4}$ radians, so that after the plate the phase difference is extended $\frac{\lambda}{2}$ in space, or $\frac{\pi}{2}$ radians, and destructive interference can occur. Again, $\tau = 2\pi$ is used [75].
Chapter 2. Theory

2.10 Light microscopy

\[ E_S = E_0 \sin(\omega t - \frac{T}{4}) = E_0 \cos(\omega t) \]  \hspace{1cm} (2.10)

\[ E_{PM} = E_0 (\cos \omega t + \phi \cos \omega t) = E_0 (1 + \phi) \cos \omega t \]  \hspace{1cm} (2.11)

The amplitude of resulting field is now dependent on the phase shift \( \phi \) which varies in space as the time spent of the light in media of different refractive index change from point to point in the sample plane. This phase change however is very small \( (\phi \ll 1) \). In order to have greater amplitude contrast the phase plate is made semi-transparent, with light transmittance \( A \), so only the S-wave amplitude will be reduced and brought to the same order of magnitude as \( E \). This yields that

\[ E_S = AE_0 \cos(\omega t) \]  \hspace{1cm} (2.12)

\[ E_{PM} = E_0 (A + \phi) \cos \omega t \]  \hspace{1cm} (2.13)

### 2.10.4 Differential interference contrast microscopy

The differential interference (DIC) microscope is also useful in biology for imaging unstained samples. The DIC microscope uses changes in the gradient (in the sample plane) of optical path difference travelled in the sample to create image contrast. This means that especially edges of objects in samples are well imaged. The optical path difference is the difference in optical path length travelled by the ordinary and extraordinary wave that started from the same polarized wave but was split by a first Wollaston prism and then rejoined by a second Wollaston prism. The optical path length is the product of the geometrical path length travelled by a light wave and the refractive index of the material in which it travels. The optical path length determines the phase of a wave. A walkthrough of the path travelled by light inside a DIC microscope will follow and will aid in the understanding the origin of the contrast in the DIC image. An illustration is offered in figure 2.14.

First light emerging from a light source is polarized by a polarizer. The polarized waves are then split by a Wollaston prism into a ordinary wave and a extraordinary wave whose polarizations are perpendicular to each other and both to the direction of the waves. Since the waves are split they will travel through different parts of the sample but the paths will go through adjacent points. Once the waves are rejoined by the second Wollaston prism their polarizations will be realigned and they will be able to interfere. The optical path difference generates a phase difference. If there is little or no phase difference the waves will constructively interfere, if not they will destructively interfere. This phase difference originates from a difference in refractive index of the material travelled through or the thickness of the sample or both and will therefore vary with the optical path difference. Therefore if the path travelled by the ordinary and extraordinary wave is very different, the resulting rejoined wave will have a changed intensity and will stand in contrast to the intensity of the rejoined wave of ordinary and extraordinary wave that travelled along similar paths. This is how the optical path difference and the contours of objects in the
2.11 Conceptual descriptions of timed and controlled dissolution

In this project, we attempted to achieve two different dissolution profiles for PVA films: timed dissolution or controlled dissolution. We wanted to use PVA films with one of these characteristics as a cell repellent surface in a cell array. We wanted a system consisting of cell adherent areas and cell repellent areas (PVA) where cells could attach on predefined designated areas. After the cells have attached the cell repellent PVA film could be removed and expose an underlying cell adhesive surface.

With timed dissolution, the PVA film should start dissolving slowly when cells in solution are introduced to the cell array. The PVA should not dissolve completely and lose its cell repellent properties before the cells have attached to the designated cell adhesive areas, a...
process that takes a few hours. In controlled dissolution, the moment of PVA dissolution is decided by an external factor, i.e. the introduction of a compound that destabilizes PVA and causes its dissolution. The PVA should therefore be completely stable in solution before the introduction of this compound.

An illustration of ideal dissolution profiles can be found on figure 1.3 on page 5, and a conceptual illustration of PVA in a cell array undergoing timed and controlled dissolution is shown on figure 5.1 on page 79.

2.12 Borate and sodium sulfate stabilization of PVA

In this project we attempted two alternative methods for stabilizing PVA films other than baking. The literature suggests that PVA hydrogels can be stabilized through cross-linking PVA chains with borate or inducing hydrogen bonds between chains by "salting out" with sodium sulfate.

2.12.1 Borate stabilization of PVA

Borate has been used to induce covalent cross-linking between PVA chains. The literature disagrees slightly about the nature of the cross-linking. Cui et.al. [78] has suggested that the active compound in this reaction is borate ion $B(OH)_4^-$, and that the cross-linking occurs at low concentration (2 mM, figure 2.15a) where two adjacent hydroxyl groups in PVA are joined by a boron bridge to two other adjacent PVA hydroxyl groups. At higher concentrations, there is no cross-linking, as the borate ions are in such abundance that majority of hydroxyl groups will already be covalently linked to a boron atom, preventing the cross-linking from happening (figure 2.15c). The pKa of the equilibrium between boric acid $B(OH)_3$ and borate $B(OH)_4^-$ is 9.2 (figure 2.15d), indicating that cross-linking is favorable in highly basic environments. The PVA cross-linking of borate is furthermore reported to be a reversible process. If glucose is added there is a competition between glucose and PVA for binding borate, in the favor of glucose (figure 2.15f). This way, the covalent cross-linking bridge of PVA can be selectively ended. Manna et.al. reports a different system where PVA is stabilized with by cross-linking PVA with borate through hydrogen bonds [79] (see figure 2.16). This process is also reversible when adding glucose to the system, as glucose competes with PVA for binding borate. These borate stabilized hydrogels are made by mixing PVA solution with borate solution.
2.12 Borate and sodium sulfate stabilization of PVA

Chapter 2. Theory

(a) $c = 2$ mM
Cross-linked PVA

(b) $c = 20$ mM
Uncross-linked PVA

(c) $c = 70$ mM
Uncross-linked PVA

\[
\text{B(OH)}_3 + \text{H}_2\text{O} \rightleftharpoons \text{B(OH)}_4^- + \text{H}^+ \quad \text{pK}_a \approx 9.2
\]

(d) Equilibrium reaction between boric acid $\text{B(OH)}_3$ and borate $\text{B(OH)}_4^-$

(e) Binding mechanism of borate to PVA

(f) Competition for borate binding between glucose and PVA

**Figure 2.15:** Cui *et al.*'s model for reversible PVA stabilization of borate cross-linking [78]. $c$ is the concentration of borate. Details are given in the text.
Figure 2.16: Manna et.al’s model for reversible PVA stabilization of borate cross-linking [79]. Details are given in the text.
2.12.2 Sodium sulfate stabilization of PVA

Highly concentrated sodium sulfate has been used to stabilize PVA through "salting out" [80, 81]. Salting out, also called antisolvent crystallization, is a technique for extracting water from a solution and for isolating the solute [82]. A concentrated aqueous salt solution is introduced and water molecules are attracted to the salt ions and flow away from the solute of interest and into aqueous salt phase. For the stabilization of PVA the salt solution extracts the remaining water molecules in the PVA film after spin coating. This allows the hydroxyl groups of PVA monomers to form hydrogen bonds between each other, hereby stabilizing the film, much like baking.

![Figure 2.17: Structure of sodium sulfate [83]](image)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Molecular formula</td>
<td>Na₂SO₄</td>
</tr>
<tr>
<td>Water solubility (20°C)</td>
<td>19.23 g/(100 mL)</td>
</tr>
<tr>
<td>Molar mass</td>
<td>142.04 g mol⁻¹</td>
</tr>
</tbody>
</table>

**Table 2.3:** Properties of sodium sulfate [83]
Chapter 3

Methods

3.1 PVA thin film solubility testing

In this section, the experimental procedure of PVA thin film solubility testing are described. We begin by explaining how we determined the dissolution properties of PVA films on wafer samples (section 3.1.1). Then we described the experimental set-up of the solubility tests (section 3.1.2). Next, we explain how the PVA film samples were prepared (section 3.1.3). Finally, we describe the set-up of the alternative approaches to baking for stabilizing PVA films (section 3.1.4).

3.1.1 Determination of dissolution properties

Here we describe how PVA film dissolution properties (whether the film is soluble or not and how quickly it dissolves) were determined. As sample substrates we used blank reflective silicon wafers (Si-wafers). The wafer samples were about $1.5 \times 1.5$ cm in area. The method was developed during the preceding project work [1]. The solubility of PVA was tested in water and in other solutions.

Qualitative visual testing

The presence of PVA thin films on Si-wafers could be immediately verified by observing interference patterns on the wafer surface. The patterns changed color according to the film thickness (examples given in figure 3.1a through 3.1g), and samples with no PVA thin film present were blank and reflective (figure 3.1h). By comparing the interference pattern colors of PVA samples before and after exposure to various solutions the thickness changes could be observed and the solubility of PVA in these could be quickly assessed.

Quantitative testing

In order to quantitatively observe dissolution rates, the samples’ film thickness was measured with different instruments in the way described below.
3.1 PVA thin film solubility testing

Figure 3.1: PVA films on Si-wafer substrate with interference patterns of different colors for different film thicknesses \( d \). Measurements were done with a reflectometer. Images were taken with a cell phone camera. Approximate wafer size: 1.5 cm \( \times \) 1.5 cm.

**Film thickness measurement in reflectometer** The reflectometer in use was a F20 model from FILMetrics. Data was processed by FILMetrics software (figure 3.2a). This reflectometer could measure film thicknesses from 70 \( \mu \)m down to 15 nm [76]. The films were exposed to wavelengths from 380 nm up to 1050 nm. Samples were placed on the reflectometer stage. Before doing measurements on the actual sample, the reflectivity of blank reference Si-wafer and the background reflectivity were measured. For semi-submerged samples (where only half of the sample was exposed to a solution) both non-dissolved areas and solution exposed areas were measured in order to have "before" and "after" thicknesses of the PVA films. For completely submerged samples only "after" thicknesses were available. The samples were inspected by eye on beforehand and only smooth areas (with a smooth even color) that were far away enough from the wafer edge (in order to not measure edge beads) were measured. Measurements were repeated on different areas in order to assure correct values. For the film thickness to be measured correctly, one needed to provide the software with the refractive index of the film. An image of the used reflectometer is shown in figure 3.2b.

**Film thickness measurement in profilometer** The profilometer in use was a Dektak 150 from Veeco Metrology. Data was analyzed by Dektak software. Before doing any measurements, scratches were made with a pointy tweezer to locally remove the PVA film all the way down to the wafer surface. The zero height level of plots was set by choosing two points corresponding to the wafer surface and then aligning the plot according to these points. Then, the software would calculate the film thickness by measuring the height difference between the film surface and the wafer surface. Usually, only single measurements were made. For semi-submerged samples, scratches were made in proximity of the interface between the solution exposed area and the non-exposed area. This way "before" and "after" film thicknesses could be measured in a single scan (left sample figure 3.3). For a sample that had been completely submerged in solution it was only necessary to do a scan over one single scratch (right sample figure 3.3). Examples of profilometer plots are shown in figure 3.4.
Chapter 3. Methods

3.1 PVA thin film solubility testing

(a) Reflectometer instrument. Legend: 1) Combined light source and detector, 2) Sample whose film thickness is measured

(b) FILMetrics software illustration [76]

Figure 3.2: Reflectometer instrument and software

Figure 3.3: Scratches on sample to be inspected in profilometer. The red arrows show the direction of profilometer scan. Left: semi-submerged sample, right: completely submerged sample.
Figure 3.4: Example of profilometer plots of PVA film samples on Si-wafer. Legend: 1) Tweezer scratches and wafer surface 2) Non-solution exposed film 3) Solution exposed and partly dissolved film 4) Artifact due to accumulation of PVA from scratching. The direction of scanning is shown by the red arrows in figure 3.3. The red and green lines are software measurement tools.
Film dissolution inspection in differential interference microscopy (DIC) When dealing with samples where it was unclear whether there was any PVA film left on the surface of a wafer after a solubility test, it was taken to a differential interference contrast (DIC) microscope. Scratches were made with a pointy tweezer on the sample surface and these were looked closer at in the microscope. For a blank PVA free wafer surface a scratch would appear as a rough mark on the surface. If PVA would be left on the surface, a change in color between the scratched and PVA free area and the untouched surroundings could be seen. Also, on the far end of the scratches, detached PVA layers would accumulate from the tweezer moving back and forth during scratching so that a clear contrast could be seen between the small PVA piles and the surroundings. This would not be observed on PVA free scratched samples.

3.1.2 Solubility test experimental set up

This section describes the experimental set up for conducting PVA thin film solubility tests. Samples of PVA thin films were prepared (as described in section 3.1.3) and exposed to the solution in which the PVA thin film solubility was to be investigated. The solubility was tested in deionized water (referred to simply as water in the report), HeLa cell medium or PBS (solutions of interest). Detailed information about the cell medium and PBS used is given in section 3.2.1 on page 38. Times of exposure to these solutions were noted $t_w$, $t_{CM}$, $t_{PBS}$ for water, cell medium and PBS respectively. Several identical PVA thin film samples were prepared (see section 3.1.3) and exposed to solutions only once for different times ranging from seconds to several hours or days. For experiments aiming to give an overview of PVA thin film dissolution properties, tests were done in ambient conditions. For experiments testing dissolution properties in the conditions of a cell micro-array, tests were done in the clean room$^1$.

Solubility tests in ambient conditions

PVA thin film samples to be solubility+ tested were lowered in a beaker containing the solution of interest at room temperature. Using a custom made device (see figure 3.5), samples could be lowered and raised from the solution quickly and solution exposure times could be decided precisely. Samples were then blow dried with an air gun. Samples were photographed with a regular cell phone camera before and after solution exposure to compare PVA interference patterns and determine solubility as explained in section 3.1.1. To be able to distinguish interference patterns, samples were imaged in front of a white wall that assure even lighting of the samples.

Clean room solubility tests

A container with the solution in which the solubility test was to be done was preheated in an oven to $37^\circ$C (see figure 3.6a). It was then poured into a Petri dish so that the liquid level was about half of the height of the wafer sample width. The Petri dish was kept in the oven and the wafers were placed in the Petri dish up against its side wall so that half the surface was covered in water and the other half stayed only in air (see figure 3.6b). For

$^1$ISO7: less than $1.0\cdot10^7$ particles larger than 0.1 microns per m$^3$
samples staying in solution for longer amounts of time (hours) it was impractical to keep only half the wafer surface exposed to the solution as the solvent might have evaporated and the liquid level could drop so that the time of exposure would become uncertain. These samples were completely submerged in solution in a Petri dish which was covered with Parafilm (see figure 3.6c). After solution exposure for a chosen amount of time, the solutions were blow dried with an air gun or a nitrogen gun.

Pictures of the samples were taken with a regular cell phone camera. The film solubility was determined both qualitatively and quantitatively as explained in section 3.1.1. For the samples where only half of the PVA surface had been exposed to solution it was possible to compare the film thickness from before and after the film dissolution test. In order to see the thin film interference patterns clearly on the photograph, weak but evenly spread light needed to come from a light source, reflect on the wafer surface and reach the camera. This was achieved by suspending a thin paper tissue over samples and under a strong light source. The tissue would dim and spread the light evenly on the sample (figure 3.6d).

**Manipulation of wafer sample images in the report**

In this report, wafer images are shown without color manipulation to show interference pattern colors as they were. The quality of the illumination of the sample and the camera focus varied some. Some images were rotated in order to show the different images of the same sample from the same angle. Some images were stretched to improve the overall figure layout.

**Vocabulary clarification: "exposure" vs. "incubation"**

During the solubility test in the results section we use different terms to refer to when samples were in solutions. "Exposure" is used for samples in solutions at room temperature. "Incubation" is used for samples in 37°C solutions.
(a) Solubility testing solution in beaker heated up to 37°C inside oven, to be poured into Petri dish before dissolution testing

(b) Semi-submerged samples placed against Petri dish wall in 37°C water bath

(c) Completely submerged samples in 37°C solution bath in Petri dish covered with Parafilm

(d) Set-up for photographing wafer samples after solubility testing

Figure 3.6: Water bath preparation, dissolution testing and photographing of PV A on Si-wafer. Legend: 1) Parafilm preventing solution from evaporating, 2) Non-solution exposed half of semi-submerged sample, 3) Water exposed half of semi-submerged sample, 4) Liquid line of solution in Petri dish, 5) Light dimming paper tissue for better imaging of samples, 6) Dissolution tested samples to be imaged

3.1.3 PVA thin film sample on wafer preparation

Here we describe how we prepared the PVA samples. First we describe how we applied PVA solution onto wafer substrates to create the films, then we describe how PVA stock solutions were made. Finally we explain how we made mixed solutions of different PVA types.

PVA thin film preparation

A PVA solution was pipetted onto Si-wafers. The wafers were then spin coated at around 3000 rpm until a stable interference pattern appeared indicating the formation of a PVA thin film (usually after 30 s). The film was then stabilized through baking or through exposure to a stabilizing solution (see section 3.1.4). Baked samples were baked for 5 minutes at 130°C unless mentioned otherwise. Prior to spin coating the wafers were cleaned. For experiments in ambient conditions, the
wafer were simple rinsed in hot water (approaching boiling temperature) dissolving all previously adhering PVA instantly. For experiments in the clean room, the wafers were plasma cleaned for 5 minutes in a PlaFemto model from Dier Electronics [84] using O₂ gas (power: 100 W, pressure: 0.6 mbar).

**PVA stock solution preparation**

The PVA stock solutions were created by dissolving PVA powder. Three different kinds of PVA powders were available. They are listed with their properties in table 3.1.

<table>
<thead>
<tr>
<th>PVA supplier</th>
<th>Molecular weight ( \overline{m_n} ) [kDa]</th>
<th>Degree of hydrolysis</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDH</td>
<td>22</td>
<td>( \geq 98% )</td>
<td>high-PVA</td>
</tr>
<tr>
<td>Sigma-Aldrich</td>
<td>30–70</td>
<td>87–90%</td>
<td>low-PVA</td>
</tr>
<tr>
<td>AppliChem</td>
<td>72</td>
<td>85–89%</td>
<td>low2-PVA</td>
</tr>
</tbody>
</table>

**Table 3.1:** List of available PVA types with molecular weight and degree of hydrolysis. The names given the different PVA (high/low) types reflect their degrees of hydrolysis. PVA from Sigma-Aldrich is simply referred to as "low-PVA" and not "low1-PVA" because this PVA type was much used in this project whereas "low2-PVA" (from AppliChem) was only used for a few experiments.

The PVA powder was weighed and poured carefully into a small glass vial. The amount of PVA needed was calculated from the relations below. \( wt\% = \frac{m_{PVA}}{m_{PVA} + m_w} \), \( m \) is mass, \( \rho \) is density, \( V \) is volume and index w designates water. The PVA powder mass needed was calculated using the following relation:

\[
m_{PVA} = \frac{wt\%}{1 - wt\%\rho_wV_w}
\]

knowing that \( m_w = \rho_wV_w \) and \( \rho_w = 1 \text{ g mL}^{-1} \). The water volumes used were around 5 and 10 mL. During pouring, the vial was placed in a water bath of over 85°C (the PVA glass transition temperature) on top of a hot plate and the solution was continuously mixed with a magnetic stirrer. Some gel clusters were usually formed during pouring but they eventually got dissolved after a certain time of stirring in hot water above the glass transition temperature. Bubbles often formed during stirring but they usually disappeared after a few hours after the PVA was dissolved. PVA stock solutions were then kept at room temperature and used for deposition on Si-wafers or on glass slips to make PVA films. The stock solutions remained homogenous over several weeks.

**Mixed PVA solution preparation**

In order to test the solubility of PVA films made of a composition of different PVA types they needed to be properly mixed. First, two stock solutions of different PVA types were selected. Then, the relative volume composition of each PVA type was decided. Usually, around 400 \( \mu \text{L} \) were made of each mixed solution as this was enough to prepare 3–5 samples and have some left as back up in case more samples needed to be prepared with the
same PVA composition. Correct volumes of each PVA type were then transferred to an Eppendorf tube using a micropipette. The new mixed solution was stirred using a vortex mixer. The solution was then placed in a hot water bath (70–90°C) for 30 minutes to 1 hour to aid the PVA chains from the different stock solutions to diffuse well and make a homogeneous solution. The set-up for the water bath was to place a wide water recipient on a hot plate, cover the top with aluminum foil or a paper tissue, poke a narrow hole in the cover and place the Eppendorf tube in the hole so that the tube lid held the tube in an upright position and the bottom of the tube containing the mixed solution would be exposed to hot water. The mixed solution was then stirred again in a vortex mixer. Finally, the solution was let to cool down to room temperature before it was to be pipetted on samples for spin coating.

3.1.4 Stabilizing soluble PVA thin films through exposure of various solutions

In the attempt to modify PVA thin film dissolution properties, room temperature water soluble PVA thin film samples were lowered into the stabilizing solution (at room temperature) with a custom made device shown in figure 3.5. After stabilizing solution exposure, the sample was blow dried with an air gun. Samples were then inspected for interference patterns to see if the film was stable in these solutions. Sample images were taken with a regular cell phone camera. After removal of the samples from the stabilizing solutions, solute would often deposit on the wafer surface resulting in visible interference patterns. To avoid misinterpreting solute induced interference patterns as present PVA thin film a small surface on the corner of the wafer sample had its covering PVA film removed (see figure 3.7) by dipping the wafer corner in hot water (in which any PVA thin film dissolves instantly). This way any interference pattern appearing on this area would be solely due to solute deposition. This area would then serve as a control reference for interference patterns due to solute deposits, and not PVA films.

![Figure 3.7: PVA thin film sample on wafer with PVA free corner. Legend: 1) PVA thin film, 2) PVA free corner. The image was taken with a cell phone camera. Approximate wafer size: 1.5 cm × 1.5 cm.](image-url)
3.2 Cell work

In this section, the details regarding cell culturing and cell experiments are explained. In this project, we used HeLa cells. All cell work was done in a sterile bench.

3.2.1 Cell culturing

The HeLa cells were cultured according to the following protocol [85]:

**Materials used**

- HeLa cells, from the Department of Cancer Research and Molecular Medicine (IKM), St. Olav’s Hospital, Trondheim University Hospital
- Sterile bench with laminar air flow (Holten LaminAir)
- HeLa cell medium, original volumes of components: 500 mL DMEM (Dulbecco’s Modified Eagle Medium 1X, Life Technologies, cat.no.: 31053), 50 mL FBS (Fetal Bovine Serum, Sigma Aldrich, cat.no.: F7524), 5 mL NEAA (MEM Non-Essential Amino Acids 100X, Life Technologies, cat.no.: 11140), 2.5 mL L-Glutamine (200 mM, Sigma Aldrich, cat.no.: G7513)
- Sterile PBS (Dulbecco’s Phosphate Buffered Saline, Sigma Aldrich, cat.no.: D8537)
- Trypsin (0.25% Trypsin-EDTA solution, Sigma Aldrich, cat.no.: T4049)
- Cell culturing flask of volume 25 cm$^3$ with CO$_2$ filter cap (TC25, VWR, cat.no.: 734-2311)

**Cell splitting**

The cell splitting procedure was done twice a week, each time seeding $5 \cdot 10^5$ cells. First, cell medium, PBS and trypsin were heated up to 37°C in water bath. Cell medium was sucked out from the cell flask of the old cell culture and replaced with 2 mL PBS evenly spread out across the cells. The PBS was then replaced with 1 mL trypsin before the flask was incubated at 37°C for 2 or 3 minutes. Next, 2 mL cell medium was added to the flask. Cells were then loosen and the solution was made homogeneous by carefully pipetting them and transferring them to a centrifugation tube. A small volume of the cell solution was extracted and cells were manually counted in a light microscope to find the cell concentration of the solution in the tube. The tube was then centrifuged at 1500 rpm.
for 5 minutes. The surfactant was then sucked out and cell medium was added to achieve a concentration of $5 \cdot 10^5$ cells/mL. $5 \cdot 10^5$ cells were transferred to a new cell culturing flask and the flask was filled with an additional 4 mL of cell medium. The new cell culturing flask was then kept in an incubator at $37^\circ C$ until the next cell splitting. If cells were needed for experiments, the remaining cells in the centrifugal tube were used.

### 3.2.2 Set up for cell experiment

**Glass slip sample preparation for cell experiments**

Cell experiments with PVA were done on thin glass slips (diameter: 13 mm). The slips were rinsed in a 1 M HCl solution and were marked on their back side (to avoid contamination on the top side) with an asymmetrical sign (*i.e.* the letter F) in order to recognize which side was up after PVA coating. PVA thin films were made by spin coating PVA solution onto the glass slips for around 30 seconds at around 3000 rpm on custom made spin coater. The samples were then baked at $130^\circ C$ for 5 minutes unless mentioned otherwise. Before seeding of cells onto the glass slips, the slips were sterilized by ethanol rinse for a few minutes. The slips were then let to be air dried before use in experiments.

**Cell repellence tests**

In order to assess cell repellent properties of PVA thin films, certain parts of the glass slips were made cell adhesive. This would be done either by microcontact printing cell adhesive polydopamine onto the PVA film surface (see section 3.3.1) or by keeping one side of the glass slip PVA free. The latter was done by placing piece of tape on one side of the glass slip, coating PVA onto the entire slip, then ripping the tape off leaving one side of the glass slip PVA free and cell adherent. After cell deposition cell adherence between the PVA covered side and PVA free side could be compared in a light microscope. Cell experiments were done in a 24 well plate. The glass slips were placed in wells and each well was filled with 0.5 mL cell medium and HeLa cells at concentration $2 \cdot 10^5$ mL$^{-1}$, thereby seeding each sample with approximately 100 000 cells. The well plate was then incubated at $37^\circ C$. In order to remove non-adhering cells from the sample surface after incubation, each well had its cell medium sucked out, rinsed with PBS and refilled with cell medium twice after 6–24 hours of incubation. Control wells both with and without glass slips were seeded with cells as well.

**Cell sample imaging and cell repellence/adhesion determination**

Cell samples were imaged after incubation of at least 24 h so cells would have time to attach and grow properly. The cells were imaged in phase contrast mode of a light microscope. Cell repellent/adhesive properties of surfaces were determined by interpreting cell morphologies. Adhered cells appear to stretch out in several directions (figure 3.8a) whereas non-adhered or repelled cells display a circular shape ((figure 3.8b)). Dividing cells also appear circular but they can be identified as cell pairs joined by a contractile ring which appears as a straight line in images (figure 3.8c). Also, when assessing cell
repellence or adhesion one compares several cells within the same image frame. It is unlikely that all cells within an image frame divide simultaneously and so that they could be misinterpreted as non-adhered cells.

![Image](image.png)

**Figure 3.8:** Example images of adhered, non-adhered and dividing cells. Images were taken in phase contrast mode. Scalebar: 25 µm.

### 3.3 Patterning of PVA films

In this section, we describe the two methods used for patterning cell adhesive areas on a cell repellent PVA film.

#### 3.3.1 Microcontact printing

Using microcontact printing, polydopamine was patterned onto PVA films. Pre-made PDMS stamps consisting of square or stripe patterns were used. The stamp was coated with polydopamine by floating it on 1 mg mL\(^{-1}\) dopamine hydrochloride solution in 10 mM tris buffer at pH 8.5. The stamp was blow dried with an air gun to leave a polydopamine film on the stamp surface. The polydopamine was then transferred by placing the stamp face down on a PVA film sample and under weight from the flow of air from the air gun for 1 minute so that even colored interference patterns emerged from the whole stamp-sample interface. The stamps were reusable and polydopamine residues from the stamp surface was removed with a sticky tape.

#### 3.3.2 Photolithography

The procedure for the photolithographic patterning of PVA thin films was as follows. First, OmniCoat [86] was spin coated onto pre-made PVA films (less than 1 mm thick) at 3000 rpm for 35 seconds and then baked at 115°C for 1 minute. This was done twice in order to facilitate the future lift-off. Next, the negative photoresist SU-8 2 [87] was spin coated onto the OmniCoat layer at 6000 rpm for 35 seconds and then baked at 95°C for 1 minute, making a resulting photoresist layer of 1 mm. For patterning in the photoresist a square grid mask consisting of 200 µm squares spaced with 400 µm was used. Samples were exposed to i-line UV light (wavelength 365 nm) in a mask aligner (KSM MJB-3 HP, Karl Süss [84]). The exposure dose of the resist was 120 mJ cm\(^{-2}\). The samples were post
exposure baked at 95°C for 1 minute. The patterns in the photoresist were then made by exposing samples to a developer (mr-Dev 600, Micro Resist Technologies [88]) for 1 minute. The result was an intact PVA film layer under an intact OmniCoat layer under a photoresist layer with square shaped holes. Next, the samples were put in a plasma cleaner (PlaFemto, Dier Electronics [84]) for 5 minutes using O₂ gas (power: 100 W, pressure: 0.6 mbar) in order to etch square holes in the PVA film but letting the thicker photoresist protect the rest of the PVA film. Finally, the remaining photoresist was removed by spraying the samples with acetone and keeping them in ultrasonic acetone bath for 1 minute.

### 3.4 Other chemicals used

In this project we used different chemicals to coat PVA films with. We used poly(acrylic acid), or PAA, mixed with PVA to destabilize PVA films and 3-aminophenylboronic acid monohydrate, or APB, to coat PVA films hoping to make them cell adhesive. Their details are given in table 3.2.

<table>
<thead>
<tr>
<th></th>
<th>Molecular weight [Da]</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(acrylic acid)</td>
<td>~ 100 000</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>3-Aminophenylboronic acid monohydrate</td>
<td>154.96</td>
<td>Sigma-Aldrich</td>
</tr>
</tbody>
</table>

*Table 3.2: Chemicals used with illustrations and properties [89]*
3.4 Other chemicals used

Chapter 3. Methods
Chapter 4

Results

This project consists of two main parts. First, we attempted to control the dissolution of readily water soluble low-PVA thin films (section 4.2). Secondly, we explored alternative methods for stabilizing PVA thin films in hope of achieving timed or controlled dissolution (section 4.3). We also explored the possibility of destabilizing dissolution resistant PVA films (section 4.4). We will begin, however, with the some simple solubility tests of the different types PVA thin films worked with.

4.1 Reference solubility tests

To start with, we tested the solubility of different types of PVA films. PVA film samples were prepared by spin coating PVA onto wafers as described in section 3.1.3 on page 35. The solubility of the films were determined by comparing the resulting interference patterns on samples before and after exposing them to water at room temperature, as explained in section 3.1.2 on page 33. The results are presented in figure 4.1. Baked low-PVA films, low2-PVA films and unbaked high-PVA films dissolved almost instantly, as can be seen by the blank samples after 10 seconds of water exposure. High-PVA films were stable in water if they were baked. Here, some PVA dissolved within the first seconds of water exposure but the film thickness stabilized quickly, as interpreted by the similar interference pattern colors of the films after 10 seconds and 2 hours of water exposure. By comparing the different PVA types in table 3.1 on page 36 we see that the water stable PVA film was the baked one that was fully hydrolyzed (degree of hydrolysis ≥ 98%) and the baked ones that had lower degree of hydrolysis were readily water soluble. There appeared to be no difference in solubility in water at room temperature or at 37°C. Therefore, for simplicity reasons, many solubility tests were done at room temperature. The room temperature soluble films were also soluble at 37°C (data not shown).
4.1 Reference solubility tests

Chapter 4. Results

Figure 4.1: Reference solubility tests in water for the films baked low-PVA, baked low2-PVA, unbaked high-PVA and baked high-PVA. Images were taken before and after water exposure. All tests were done in room temperature water (except for the bottom image samples). Water exposure times were 10 s for all samples except for: *) 1 min, **) 2 h, ***) 3 h. For the differences between the PVA types, see table 3.1 on page 36.
4.2 Controlled dissolution of water soluble low-PVA films

After testing the solubility of different types of PVA films in water we wanted to know if the dissolution properties were the same in cell compatible media. These tests suggested that low-PVA films, which are readily water soluble, were not completely soluble in cell medium (section 4.2.1). Therefore we hoped that low-PVA films would have cell repellent properties, but the subsequent cell repellence tests failed to demonstrate so (section 4.2.2). Finally, during new solubility tests it was discovered how the initial promising data suggesting the dissolution resistance of low-PVA in cell medium was misinterpreted (section 4.2.3). The possibilities for using photolithography to pattern low-PVA were also investigated (section 4.2.4).

4.2.1 Comparing dissolution properties of low-PVA thin films in cell medium, PBS and ethanol

We tested the solubility properties of high-PVA and low-PVA in cell medium to see if they were the same as in water. Low-PVA films are soluble in room temperature water (and at 37°C) and high-PVA is dissolution resistant in 37°C water (see figure 4.1). PVA films were baked and prepared according the methods described in section 3.1.3 on page 35. Solubility tests were done in the clean room according to the methods described in section 3.1.2 on page 33 by incubating PVA thin film samples in cell compatible media at 37°C. The concentrations of the PVA stock solutions used were 10% (low-PVA) and 5% (high-PVA).

The results of the solubility tests in cell medium were surprising. For the water soluble low-PVA films there were consistent remaining interference patterns on the wafer sample surfaces after cell medium incubation indicating the presence of a remaining film (table 4.1). The interference patterns and the measured film thicknesses were similar after 10 minutes and after 21 hours, although these films were much thinner than before incubation (dropping from around 700 nm to 100 nm). We therefore hypothesized that low-PVA films were not completely soluble in cell medium and that the low-PVA films stabilized after a few minutes of incubation. High-PVA films seemed almost unaffected by the cell medium incubation (table 4.2). Before and after cell medium incubation the interference pattern colors on the high-PVA films seemed to be quite similar. The measured film thicknesses after incubation were almost the same as the original film thickness (around 170 nm), sometimes even thicker. This was contrary to previous solubility tests in water where there was a quick marked drop in film thickness after water incubation. Still, in both water and cell medium high-PVA film seemed very stable and insoluble.

The possibility that low-PVA films were not completely soluble in cell medium was interesting. We therefore wanted to know if a particular ingredient of cell medium was responsible for this, and if changing the concentration of certain ingredients could tune the dissolution properties of low-PVA films. The main ingredient of the cell medium used was DMEM (see section 3.2.1 on page 38 for the list of cell medium ingredients). If the results of solubility tests in DMEM were the same as in cell medium it would indicate that DMEM probably was the reason behind the surprising remaining interference patterns on low-PVA film samples after cell medium incubation. The solubility tests of low-PVA in...
DMEM (table 4.3) indicated that this was indeed the case. Even after long incubation times of 6 hours interference patterns were still clearly visible. Next, we wanted to know how low-PVA films responded to PBS incubation. The wafer samples shown in table 4.4 suggested that low-PVA thin films appeared to have very similar dissolution properties in PBS as in water. After 10 seconds of incubation in PBS there were still visible interference patterns but they were completely gone after 10 minutes in PBS. The reflectometer measured a remaining film of less than 30 nm on samples incubated for 10 minutes or longer. Tweezer scratches on samples before and after PBS incubation were compared in a DIC microscope. Pre-incubated samples showed a continuous film (figure 4.2a) in contrary to incubated samples that showed only spotted areas (figure 4.2b), most likely deposited salt crystals from the PBS solution and no continuous film.

The most obvious interpretation of the resulting interference patterns and sample measurements of the low-PVA solubility tests in cell medium and in PBS were that low-PVA thin films were not completely soluble in cell medium but were in PBS. Immediately, we hoped that it could be possible to control the dissolution of low-PVA films in a cell array by first exposing the films to cell medium where they would not dissolve completely and then replace the cell medium with PBS dissolving PVA but preserving cells. We therefore wanted to know next if cell medium incubation followed by PBS incubation would dissolve low-PVA films as well, hoping that the cell medium incubation would not stabilize the film enough to make it insoluble in PBS.

Low-PVA thin film samples were incubated in cell medium for 4.5 h before they were taken out and directly incubated in a PBS solution for different amounts of time, without blow drying samples in between. The results are shown in figure 4.3. Control samples that were not incubated in PBS but only in cell medium showed a visible interference pattern, as before. Samples that were later incubated in PBS were blank already after 40 seconds. Inspection of tweezer scratches on the latter samples in a DIC microscope showed the presence of salt crystals on the wafer surface (figure 4.4a). These were easily removed by a one second long dip in water (figure 4.4b). Therefore there was no indication of PVA films left on the surface of samples incubated in cell medium, then in PBS.

In order to assure that low-PVA thin films could be used as a cell repellent substrate in a cell array, it had to be withstand ethanol sterilization. So solubility tests in ethanol were conducted. Samples were left to be air dried in the clean room. Judging by interference pattern colors, 5 minute long ethanol (figure 4.5b) exposure had no visible effect in the PVA film thickness but a 2.5 hours long exposure did (figure 4.5c). Subsequent solubility tests in cell medium followed by PBS (figure 4.6) did not appear to have different results than previous ones without initial ethanol rinse.

**Summary of solubility testing of low-PVA thin films in cell medium and PBS**

Investigating PVA thin film dissolution properties in cell medium, PBS and ethanol yielded surprising results: most notably remaining interference patterns on low-PVA samples after cell medium incubation. No interference patterns were seen on PBS incubated low-PVA samples, nor on samples incubated in cell medium followed by PBS incubation. The main hypothesis was that low-PVA film did not dissolve completely in cell medium but did so quickly in PBS. This meant that it could be possible to control the moment of dissolution
Chapter 4. Results

4.2 Controlled dissolution of water soluble low-PVA films

<table>
<thead>
<tr>
<th>$t_{CM}$</th>
<th>Film thickness [nm]</th>
<th>Image of sample</th>
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<td></td>
<td>Reflectometer</td>
<td>Profilometer</td>
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<td>240</td>
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<td>10 s</td>
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<td>199</td>
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<td>10 min</td>
<td>119</td>
<td>152</td>
</tr>
<tr>
<td>10 min</td>
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<td>67</td>
</tr>
<tr>
<td>4 h</td>
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<td>112</td>
</tr>
<tr>
<td>4 h</td>
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<td>118</td>
</tr>
<tr>
<td>21 h</td>
<td>75*</td>
<td>93</td>
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Table 4.1: Solubility tests of low-PVA in cell medium. Film thicknesses before solubility testing (averaged over three random samples): 687 nm (reflectometer) and 668 nm (profilometer). Images were taken before and after cell medium incubation. $t_{CM}$ is the cell medium incubation time. *) Reflectometer measurements with goodness of fit lower than 0.5.

<table>
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<tr>
<td>10 s</td>
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<tr>
<td>10 min</td>
<td>179</td>
<td>214</td>
</tr>
<tr>
<td>4 h</td>
<td>155</td>
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</tr>
<tr>
<td>4 h</td>
<td>146</td>
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</tr>
<tr>
<td>21 h</td>
<td>156</td>
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</tr>
<tr>
<td>21 h</td>
<td>174</td>
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Table 4.2: Solubility tests of high-PVA in cell medium. Film thicknesses before solubility testing (averaged over three random samples): 179 nm (reflectometer) and 155 nm (profilometer). Images were taken before and after HeLa cell medium incubation. $t_{CM}$ is the cell medium incubation time. *) Reflectometer measurements with goodness of fit lower than 0.5.
## 4.2 Controlled dissolution of water soluble low-PVA films

### Chapter 4. Results

**Table 4.3:** Solubility tests of low-PVA in DMEM. Film thicknesses before solubility testing (averaged over three random samples): 734 nm (reflectometer) and 709 nm (profilometer). Images were taken before and after DMEM incubation. $t_{DMEM}$ is the DMEM incubation incubation time. *) Reflectometer measurements with goodness of fit lower than 0.5.

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<tr>
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<td>86*</td>
<td>84</td>
</tr>
<tr>
<td>6 h</td>
<td>83*</td>
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</tbody>
</table>

**Table 4.4:** Solubility tests of low-PVA in PBS. Film thicknesses before solubility testing (averaged over three random samples): 727 nm (reflectometer) and 711 nm (profilometer). Images were taken before and after PBS incubation. $t_{PBS}$ is the PBS incubation incubation time. *) Reflectometer measurements with goodness of fit lower than 0.6.

<table>
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<td>98</td>
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</tr>
<tr>
<td>6 h</td>
<td>30*</td>
<td>N/A</td>
</tr>
<tr>
<td>6 h</td>
<td>18*</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Chapter 4. Results  4.2 Controlled dissolution of water soluble low-PVA films

Figure 4.2: DIC image of tweezer scratches of low-PVA film samples incubated in PBS. Scale bar: 50 µm. To the left we see scratches in the PVA film, to the right darker areas around the scratches appear to be salt crystals.

Figure 4.3: Solubility tests of low-PVA in cell medium followed by PBS. Cell medium incubation time: 4.5 h. Interference patterns were still visible after incubation in cell medium, but disappeared after short PBS incubation times (c,d).

Figure 4.4: DIC image of tweezer scratches of low-PVA film samples after incubation in cell medium followed by incubation in PBS. Scalebar: 50 µm. The water rinse appeared to remove the salt crystals visible on the left image.
4.2 Controlled dissolution of water soluble low-PVA films  

Chapter 4. Results

Figure 4.5: Solubility testing of low-PVA films in ethanol. Ethanol exposure times are indicated. 5 min: no/little effect on films, 2.5 h: films became more uneven.

Figure 4.6: Solubility testing of ethanol rinsed low-PVA films in cell medium followed by PBS treatment. Within each subfigure – left: after cell medium incubation (2.5 h), right: after PBS incubation (7 min). Ethanol exposure times are indicated. For both ethanol exposure times interference patterns were visible after cell medium incubation but not after PBS incubation.

of a cell repellent PVA film in a cell array by simply changing the cell medium (in which the cells grow) to PBS, thereby quickly clearing new cell adhesive areas. In order to pursue this hypothesis, cell adherence experiments on low-PVA films were done.
Chapter 4. Results  

4.2 Controlled dissolution of water soluble low-PVA films

4.2.2 Cell repellence tests on low-PVA

In this section, we follow up the results from section 4.2.1 suggesting that water soluble low-PVA films did not dissolve in cell medium but did in PBS. We wanted to know whether low-PVA films in cell medium were cell repellent. Sample preparation and cell repellence tests were done according to the methods described in section 3.2.2 on page 39. Two types of samples were prepared: plain PVA films and PVA films microcontact printed with polydopamine.

Cell repellence tests on plain low-PVA films

We wanted to test the cell repellent properties of low-PVA films and see whether PBS incubation would increase cell adherence. Four different experiments were conducted on low-PVA films on glass slips. Cells were always seeded in cell medium. The results are shown in figure 4.7 (more images shown in appendix and figures 7.1 through 7.4) and the four different experiments are explained below:

(a) Samples were directly seeded with cells to assess cell repellence of low-PVA in cell medium.
(b) Samples were incubated in cell medium for 1 h, before the medium was sucked out and cells were seeded to see whether changing cell medium would have any effect on cell repellence.
(c) Samples were first incubated in PBS for 1.25 h, before the PBS was sucked out and cells were seeded to see whether cell adherence these samples would be more cell adherent than the ones only incubated in cell medium.
(d) Samples were incubated in cell medium for 1 h and then in PBS for 1.25 h before the PBS was sucked out and cells were seeded to simulate all the conditions a cell array would go through.

The cell repellent properties of the low-PVA surfaces incubated only in cell medium appeared to be poor. After 24 hours of incubation with cells, although many cells appeared to be floating, there were quite a few attached cells (figure 4.7a, left). After 72 hours there was no longer any indication that the surface might have been cell repellent earlier were still repellent as the cells clearly had attached and proliferated (a, right). Similar things were observed for the samples incubated in cell medium once before incubation with cells (b). The initially PBS incubated samples did not seem to be cell repellent after 24 hours of incubation with cells, as expected, and after 72 hours cells had proliferated (c). This was also the case for the samples incubated incubated in cell medium and PBS before cell incubation (d).

The expected greater cell repellence of samples that had been only been incubated in cell medium compared to those which had been incubated in PBS was not easy to see. What was certain was that after 72 hours whatever cell repellent properties that may or may not have existed for only cell medium incubated samples were at this point absent. So low-PVA thin films were clearly not long term cell repellent like high-PVA thin films have been reported to be [15], but the short term cell repellence was difficult to assess.
Figure 4.7: Cell repellence test of low-PVA thin films. Images are taken in phase contrast mode. The legend indicates the different experiments explained by the list on page 51. Control areas were PVA free surfaces on glass. Scale bar: 100 µm. Incubation times are noted. See the text for interpretation.
Cell repellence tests of polydopamine microcontact printed low-PVA thin films

We wanted to better visualize the cell adherence contrast between intended cell repellent low-PVA film surfaces and cell adhesive surfaces. This way we could determine the short term cell repellent properties of low-PVA films. The improved contrast was achieved by microcontact printing cell adhesive polydopamine onto low-PVA thin films. The microcontact printed pattern size were at the order of tens of microns so the cell adherence/repellence contrast should be visible within a single microscope image. The printed patterns were either squares or stripes. Already confirmed cell repellent high-PVA film control samples were prepared so cell repellence of low-PVA and high-PVA films could be compared. The samples were incubated for 24 hours before they were imaged. The results presented in figure 4.8 showed no sign of cell repellence for the low-PVA samples. When comparing the low-PVA sample control to the high-PVA control it appeared as though the cells on the low-PVA samples adhered at random location without being affected at all by the initial polydopamine patterning. The high-PVA controls showed clear cell adherence/repellence contrasts although "roof collapse" of the stamp during printing occurred. Either the low-PVA films and the polydopamine patterns were absent or the low-PVA films had no cell repellent properties.

4.2.3 Solubility tests of low-PVA films of different concentrations

The cell repellence tests of low-PVA films were unsuccessful. The concentration of the PVA stock solution used for preparing these films was a weight percentage of 10%, so it was set out to see whether changing the concentration of PVA solutions, thereby changing the thickness of the resulting films after spin coating, would have any effect on film dissolution properties. Samples made from low-PVA solutions of 5%, 3.3% and 1% were prepared with standard preparation parameters. Solubility tests in cell medium were conducted, the results are shown in figure 4.9. The different colors of the interference patterns of samples before cell medium incubation indicated lower film thicknesses as the concentrations of the initial solutions dropped. After cell medium incubation however the interference pattern colors of the samples all looked alike indicating similar film thicknesses. This was surprising as the initial film thickness was expected to have an impact on the film thickness after cell medium incubation. The interference patterns of the 1% low-PVA sample before incubation were almost to week to be seen, indicating a film thickness well below 50 nm (comparing it to the sample in figure 3.1g on page 30). After cell medium incubation, the film thickness appeared to be higher as the interference pattern got much clearer. This led to the hypothesis that some cell medium solute might deposit on top of the sample surface during blow drying of the sample after incubation. A control experiment using a blank wafer incubated in cell medium was therefore conducted (figure 4.10). After only 20 seconds of incubation in cell medium and subsequent blow drying the resulting interference pattern had the same color as all the other samples that had been coated with various thicknesses of low-PVA films. The immediate conclusion drawn was that the observed interference pattern observed on low-PVA thin film samples on wafers after cell medium incubation was the due to cell medium solute deposition and not, as previously hypothesized and assumed, to a thinner remaining undissolved low-PVA film.
Figure 4.8: Cell repellence test of polydopamine microcontact printed low-PVA thin films. Images were taken in phase contrast mode. Scale bar: 100 µm. Images were taken after 24 hours of incubation. Legend: a) stripe patterned high-PVA control, b) square patterned low-PVA film, c,e) stripe patterned low-PVA film, d,f) stripe patterned low-PVA film. The cell adherent areas surrounding the squares in image a and in between the thicker stripes in image b were due “roof collapse” during microcontact printing (explained in section 2.6 on page 15). Low-PVA films showed no sign of cell repellence. See the text for further explanation.
Chapter 4. Results

4.2 Controlled dissolution of water soluble low-PVA films

Figure 4.9: Solubility test of low-PVA films made from solutions of different concentrations in cell medium. Concentrations are indicated in the figure as weight percentages. Top images: before cell medium exposure, bottom images: after cell medium incubation. After incubation all the samples displayed similar interference patterns.

Figure 4.10: Blank wafer incubated in cell medium for 20 s showing the same interference patterns as the cell medium incubated low-PVA film samples in table 4.1 and figure 4.9.

4.2.4 Low-PVA thin film patterning with photolithography

An alternative approach to microcontact printing polydopamine onto PVA to create cell adhesive and cell repellent areas is to do photolithography on PVA films. We wanted to know whether low-PVA films were compatible with photolithography. The patterning of the low-PVA films was done according to the methods described in section 3.3.2 on page 40. The films prepared were made from solutions of weight percentage 5%, 3.3% and 1%. The results shown in figure 4.11 and 4.12 indicate that well defined shapes can be made using photolithography on low-PVA films. The 1% film was accidentally exposed to an aqueous solution for a few seconds. This might explain the less well defined patterns.
4.2 Controlled dissolution of water soluble low-PVA films

4.2.5 Summary of controlling the dissolution of low-PVA films with cell medium and PBS

The initial solubility tests of low-PVA films suggested that the water soluble low-PVA films were not completely soluble in cell medium, judging by the remaining interference patterns on the wafer samples after cell medium incubation (table 4.1). No interference patterns were seen on low-PVA samples after PBS incubation, indicating its complete solubility in PBS (table 4.4). We therefore hoped it could be possible to achieve controlled dissolution of low-PVA in a cell array system: the film would be stable in cell medium, and replacing the cell medium PBS would quickly dissolve the film. Low-PVA films did not appear to be cell repellent, however (figure 4.8). We then discovered why: low-PVA films dissolved in cell medium. The reason we initially assumed the contrary was the presence of interference patterns on low-PVA wafer samples after cell medium incubation. A control experiment where a blank, PVA free wafer was incubated in cell medium showed that these
interference patterns originated from the cell medium itself (figure 4.10). What we thought to be a thin, stabilized low-PVA film was actually only cell medium solute deposited on the wafer sample during blow drying after cell medium incubation. We also showed the compatibility of low-PVA with photolithographic patterning, but unless we find a way to stabilize low-PVA films, this will have no applications. We therefore conclude: controlled dissolution of low-PVA films with cell medium and PBS cannot be achieved, like we hoped, because low-PVA films were soluble in cell medium, after all.
4.2 Controlled dissolution of water soluble low-PVA films

Figure 4.12: Profilometer scans of photolithography patterned squares on low-PVA films. Concentration of initial PVA solution before spin coating is indicated for each row. The red and green lines are software measurement tools. The spikes on the graphs are artifacts on the sample. The height drop in the 5% sample is a measurement error often encountered when doing profilometer measurements across distances approaching 1000 µm.
4.3 Stabilizing readily soluble PVA thin films

In a previous project, we established that tuning baking temperatures of PVA simply resulted in either dissolution resistant films or readily soluble films in [1]. We therefore wanted to investigate new methods for stabilizing PVA thin films. We hoped that with these methods we could achieve either timed dissolution or controlled dissolution of PVA thin films. The approaches taken presented in this section were inducing cross-linking between PVA chains with a borate solution (section 4.3.1), inducing hydrogen bond formation between PVA chains with a sodium sulfate solution (section 4.3.2) and exposing PVA films to a mixture of borate and sodium sulfate (section 4.3.3). The attempted stabilizing of films was done according to the methods described in section 3.1.4 on page 37. All solubility tests were done in room temperature water by interpreting interference patterns according to the methods described in section 3.1.2 on page 33.

4.3.1 Stabilizing PVA films in borate solutions

We wanted to know if it was possible to stabilize PVA thin films by exposing them to a borate solution by inducing cross-linking between PVA chains. To begin with, we used a saturated borate solution (673 mM) at pH 9.2. The results of the solubility tests of baked low-PVA (10%) and low2-PVA (5%) films exposed to a saturated borate solution for 2 minutes are shown in figure 4.13 and 4.14, respectively. Low-PVA and low2-PVA thin films were both completely dissolved after 5 minutes of water exposure, but some remained after 1 minute, indicating that the dissolution process was somewhat slowed down compared to films that had not been borate exposed (see figure 4.1). The remaining interference patterns on the 1 minute water exposed samples are interpreted to be PVA films, and not borate residue. This is due to the resemblance of the borderline PVA covered area/PVA free areas between the samples before borate exposure and the samples after water exposure. The PVA free areas before borate exposure were also interference pattern free after the 1 minute water exposure when other areas still displayed interference patterns.

We then tried to stabilize unbaked high-PVA, as baked high-PVA is very stable in water but unbaked (or baked at low temperature) high-PVA dissolves in water within seconds. As is shown in figure 4.15, unbaked high-PVA films were not stabilized at all by the 1.3 hour long borate exposure. The film was completely gone after only 15 seconds in water. Contrary to baked low-PVA and low2-PVA films, unbaked high-PVA films are probably not even stable in the borate solution. Before borate exposure (figure 4.15a) the sample had some blank PVA free areas. However after borate exposure the interference patterns covered the entire sample (figure 4.15b). These interference patterns were very similar to the ones seen on the PVA free control sample (figure 4.15d) and it was therefore concluded that the sample on figure 4.15b is PVA free as well. On the other hand, borate exposed and baked high-PVA films were stable in water (figure 4.16), just like non borate exposed high-PVA [1]. Although some of the film dissolved instantly in water, the similar interference patterns after 1 minute and 5 minutes indicated that the film stabilized already after 1 minute.

We then wanted to know if mixed solutions made from dissolution resistant high-PVA and readily water soluble low-PVA would behave differently if stabilized in borate. Previ-
4.3 Stabilizing readily soluble PVA thin films

Chapter 4. Results

Figure 4.13: Solubility test of baked low-PVA films stabilized in saturated borate. Legend: a,b) After bake, c,d) after borate exposure, e) water exposure time: 1 min, f) water exposure time: 5 min. Borate: $c = 673$ mM, pH = 9.2, borate exposure time: 2 min. The films were not dissolved by the borate solution, complete dissolution happened within a few minutes.

Figure 4.14: Solubility test of baked low2-PVA films stabilized in saturated borate. Legend: a,b) After bake, c,d) after borate exposure, e) water exposure time: 1 min, f) water exposure time: 5 min. Borate: $c = 673$ mM, pH = 9.2, borate exposure time: 2 min. The films were not dissolved by the borate solution, complete dissolution happened within a few minutes.

Figure 4.15: Solubility test of unbaked high-PVA film stabilized in saturated borate. Legend: a) after bake, b) after borate exposure, c) water exposure time: 15 s, d) Blank wafer after borate exposure. Borate: $c = 673$ mM, pH = 9.2, borate exposure time: 1.3 h. The film appeared to be dissolved by the borate solution.
4.3 Stabilizing readily soluble PVA thin films

Previously, tuning PVA thin film dissolution properties with various relative concentrations of these mixes was attempted but without satisfying results. The films would either dissolve completely within seconds or a minute or drop to a stable thickness within the same time frame [1]. We mixed high-PVA and low-PVA at relative concentrations ranging from 1:1.5 to 1:8 (high-PVA:low-PVA) to include the transition of relative concentrations between stable films and soluble films that was previously established [1]. The mixed solutions were made from 5% high-PVA and 5% low-PVA. First, the long term dissolution properties of baked, borate stabilized mixed films with relative concentration 1:2, 1:4 and 1:8 were investigated. After 16 hours of water exposure, the 1:2 film was partly dissolved but still present on the wafer surface, but the 1:4 and 1:8 films were completely gone (figure 4.17). The span of relative concentrations of PVA types was then narrowed down to 1:1.5, 1:2 and 1:4 where the films might be not completely soluble. Shorter dissolution times were tested (figure 4.18). After 1 minute, none of the films were gone, but after 10 minutes only the 1:1.5 film remained. Comparing the thin film dissolution rates with what was previously achieved without borate stabilization, the dissolution process before complete dissolution or stabilization of the film appeared to be a bit slower. However there was no indication that dissolution continued after a few minutes.

It was then investigated whether the borate stabilization changed the long term solubility of the PVA thin films. Baked 1:1.5 and 1:2 mixed solutions were prepared and half of the samples were exposed to borate. Figure 4.19 shows that after 3 hours in water interference patterns for both the borate stabilized and the non-borate stabilized 1:1.5 films there were equally thin remaining films present. For the 1:2 films, both the borate stabilized and the non-borate stabilized ones were completely dissolved. This indicated that the borate exposure did not render normally soluble mixed PVA films insoluble, nor vice versa.

Exposing soluble PVA films to saturated borate solutions slowed the PVA dissolution rates some but not nearly enough for timed dissolution to occur after a few hours. Nor did any borate treatment completely stabilize PVA films so maybe controlled dissolution to occur. The literature suggests that the cross linking occurs at lower concentrations [78], so it was attempted to stabilize water soluble PVA films with lower concentration borates solutions. First, baked low-PVA thin films were attempted to be stabilized with borate concentra-

Figure 4.16: Solubility test of baked high-PVA films stabilized in saturated borate. Legend: a,b) After bake, c,d) after borate exposure, e) water exposure time: 1 min, f) water exposure time: 5 min. Borate: $c = 673 \text{ mM}$, $pH = 9.2$, borate exposure time: 2 min. The films were dissolution resistant.
4.3 Stabilizing readily soluble PVA thin films

Chapter 4. Results

Figure 4.17: Long solubility test of baked mixed PVA films stabilized in saturated borate. Relative concentrations (high-PVA:low-PVA) are indicated above the images. Borate: $c = 673\, \text{mM}, \text{pH} = 9.2$, borate exposure time: 2 min. Water exposure time: 16 h. Some of 1:2 the film appeared to remain, the 1:4 and 1:8 films were completely dissolved.

Figure 4.18: Short solubility test of baked mixed PVA films stabilized in saturated borate. Relative concentrations (high-PVA:low-PVA) are indicated above the images. Top images: before borate exposure, bottom images: after borate and water exposure (water exposure times, left: 1 min, right: 10 min). Borate: $c = 673\, \text{mM}, \text{pH} = 9.2$, borate exposure time: 2 min. After 1 min in water, complete dissolution or stabilization of film thickness had not yet occurred for any films.

Concentrations ranging from 1 mM to 100 mM at pH 9.2. As the second row images of figure 4.20 shows, the low borate concentrations simply dissolved the PVA films. The higher the concentration was, the stronger the interference pattern seemed to be after borate exposure. The interference patterns could simply be due to borate depositions, however. After a 1 minute water exposure there was no PVA left indicating that the film was not stabilized at all. Then we attempted to stabilize unbaked high-PVA with low concentration borate (figure 4.21). Again, the low concentration borate solutions dissolved the films instead of stabilizing them. The borate exposed baked control sample was stable (4.21 left sample), just like non-borate exposed counterparts. It was then attempted to increase the pH of low borate concentration in order to increase the concentration of what literature suggests to be the active compound in PVA cross linking. A 50 mM borate solutions at pH 10.0 was prepared and unbaked high-PVA film samples were exposed to them for 1 minute. Again, the films dissolved during borate exposure (figure 4.22).
Chapter 4. Results

4.3 Stabilizing readily soluble PVA thin films

**Figure 4.19:** Comparing long term solubility of baked borate stabilized and non-borate stabilized mixed PVA films. Relative concentrations (high-PVA:low-PVA) are indicated above the images. Top images: Non borate stabilized, bottom images: borate stabilized Legend: a) 1:1.5, b) 1:2 (relative concentration high-PVA:low-PVA), 1) no bake 2) bake. Borate (if exposed): c = 673 mM, pH = 9.2, borate exposure time: 2 min. Water exposure time: 3 h. The borate exposure did not change whether films would reach a stable film thickness or dissolve completely.

**PVA hydrogel formation in borate solution using PVA solution**

Since we were unable to show that borate was able to stabilize water soluble PVA films, we wanted to test whether larger stable PVA hydrogels could be made in a borate solution, as a proof of concept experiment. A 5% high-PVA solution was pipetted into a borate solution of pH 10 at various concentrations, as described in section 3.1.4 on page 38. The results are shown in figure 4.23. Borate solutions of 100 mM and 50 mM gave stable and solid hydrogels. The hydrogel was marked with a pen to show the firmness of the hydrogel. 25 mM borate solutions gave a soft hydrogel that was difficult to mark with a pen. In 10 mM solutions, a hydrogel was formed but it was not stable enough to be extracted from the solution with a tweezer. The conclusion however is that making PVA hydrogels from low concentration borate solutions is possible. We also made hydrogels in saturated borate shown in figure 4.24. Hydrogels made with high-PVA were firm contrary to the very soft hydrogels made from low-PVA (5%). We wanted to know whether these hydrogels were soluble in water or in glucose, as glucose is reported to break the cross-linking between PVA chains [78, 79]. The gels were incubated for 1 hour at 37°C in water and in 300 mM glucose. The gels did not dissolve instantly but after 1 hour in water or in glucose no gels could be seen and all gels had clearly dissolved (table 4.5).

**Cell adhesion tests of aminophenylboronic acid coated PVA films**

We just showed that borate can be used to make PVA hydrogels. This proves that borate at both high and low concentrations can interact with PVA and stabilize it. We therefore attempted to coat baked high-PVA films with 3-aminophenylboronic acid monohydrate (APB), with one boronic acid group that could bind to the PVA chains and one amine
4.3 Stabilizing readily soluble PVA thin films

Chapter 4. Results

Figure 4.20: Solubility test of baked low-PVA films stabilized in low concentration borate. Borate concentration is indicated above the images. Top images: Before borate exposure, middle images: after borate exposure, bottom images: after water exposure (1 min). Borate: pH = 9.2, borate exposure time: 1 min. The low concentrated borate solutions dissolved the films.

group facing upwards that would be cell adhesive. The idea behind coating PVA with APB is that cells would be able to adhere to APB but, hopefully, an eventual glucose treatment could compete with PVA in binding the APB and thereby releasing the APB into the solution and the adhering cells with them. To test the possibility of this idea we conducted a cell adherence test of APB coated, baked high-PVA films. The results in figure 4.25 after 24 hours of incubation show that both the APB coated samples and the high-PVA control samples appear to be very cell repellent, as the visible cells only appear to be floating and not adhering to the surface. This indicates that probably APB did not adhere well to baked high-PVA in cell medium. If cell APB were to be present on the sample surfaces it could indicate that APB is not cell adhesive as assumed. Either way, APB coating of high-PVA films did not show the desired cell adherence and we did not pursue this idea.
Chapter 4. Results

4.3 Stabilizing readily soluble PVA thin films

**Figure 4.21:** Solubility test of unbaked high-PVA films stabilized in low concentration borate. Borate concentration is indicated above the images. Top images: Before borate exposure, middle images: after borate exposure, bottom images: after water exposure (1 min). Borate: pH = 9.2, borate exposure time: 1 min. The low concentrated borate solutions dissolved the films.

![Baked control](image)

**Figure 4.22:** Solubility test of unbaked high-PVA films stabilized in 50 mM borate at pH 10.0. Borate exposure time: 1 min. The low concentrated borate solutions dissolved the films.

![Images](image)

**Table 4.5:** Solubility test of saturated borate induced PVA-hydrogels in water and glucose. Incubation temperature: 37°C. Glucose concentration: 300 mM.
4.3 Stabilizing readily soluble PVA thin films

Chapter 4. Results

Figure 4.23: Low concentration borate induced high-PVA hydrogel formation. Borate: pH = 10. The borate concentration is indicated. The water control droplet on top of the pen marked background is for comparison with the pen marked hydrogels. The PVA gels and the water droplet are about 0.5 cm across.

Figure 4.24: Saturated borate induced hydrogels made from both high-PVA and low-PVA. Borate: pH = 10.
Figure 4.25: Cell adherence test of aminophenylboronic acid coated high-PVA films. Images are taken in phase contrast mode. The control sample is a uncoated baked high-PVA film. Scale bar: 100µm. Incubation time: 24h. The APB coated sample was cell repellent like the control sample, indicating that APB probably did not adhere well to the underlying PVA film.
4.3 Stabilizing readily soluble PVA thin films

4.3.2 Stabilizing PVA films in sodium sulfate solution

We then tried to stabilize PVA thin films by exposing them to a sodium sulfate solution. Sodium sulfate treatment induces hydrogen bonding in PVA films by extracting out water molecules in the film, so we chose to use unbaked high-PVA films, as baked high-PVA films already are stabilized through hydrogen bond formation. A 1 M sodium sulfate solution and a 5% high-PVA stock solution were used. As figure 4.26 shows unbaked high-PVA films were stable in the sulfate solution. Salt residues could be seen on the PVA film samples but were easily distinguishable from the PVA film as smaller flakes on the sample surface. The solubility tests summarized in figure 4.27 show that the time high-PVA thin films spent in sodium sulfate solutions affected its solubility. Sulfate exposure times shorter than 20 minutes resulted in unstable films that dissolved completely within a few minutes. Sulfate exposure times longer than 30 minutes resulted in stable films that remained stable after several hours in water. Films exposed in sulfate for around 25 minutes appeared to be in the transition between water soluble and water stable films. For these films, after 30 minutes of water exposure, some had dissolved and some had not. The overall impression of the solubility tests is that they dissolved completely or reached a stable thickness within the first 6 minutes of water exposure. We also tried to see if low-PVA could be stabilized in sulfate (figure 4.28). Baked and unbaked low-PVA-films were both intact after 1 hour and 2 hour long sulfate exposures, but were completely dissolved after 1 minute in water. Surprisingly though, the dissolution of both baked and unbaked sulfate stabilized low-PVA films seemed to be slowed down by the sulfate exposure compared to non-sulfate exposed films. The sulfate exposed films were not completely dissolved after 10 seconds in water but non-sulfate exposed baked films were (see figure 4.1).

![Figure 4.26: Verification of stability of unbaked high-PVA in sodium sulfate solution. Exposure times were 30 min (triangle sample), 2.5 h (square sample) and 2 min (control). The control sample was PVA free.](image)

Cell repellence test of sodium sulfate stabilized PVA films

Since stabilizing unbaked high-PVA with a long sodium sulfate exposure was the only way we were able to stabilize PVA films with other than baking, we wanted to see if these films also were cell repellent. We therefore exposed unbaked high-PVA films in a 1 M sodium sulfate for 2 hours and performed microcontact printing of polydopamine onto the PVA films. Regular cell repellence tests were then conducted, the results are summarized in figure 4.29. It can be seen that these PVA films are indeed cell repellent, judging by the successfully polydopamine patterned sample. The sulfate stabilized PVA films detached, however, on most samples, indicating that the sulfate stabilized films did not attach well.
to the glass substrates.
4.3 Stabilizing readily soluble PVA thin films

Figure 4.27: Solubility test of unbaked high-PVA stabilized in sodium sulfate for various lengths of time. Color fill legend: black: water exposure time, grey: sulfate exposure time.
4.3 Stabilizing readily soluble PVA thin films

Figure 4.28: Solubility test of baked and unbaked low-PVA stabilized in sodium sulfate. Left: before sulfate exposure, middle: after sulfate exposure, right: after water exposure. Sulfate: $c = 1$ M, sulfate exposure time: 1 h. Water exposure times are indicated next to the samples. The time spent in sulfate solution affects the solubility of the films. Low-PVA films were stable in sodium sulfate but the films remained soluble after sulfate exposure.

Figure 4.29: Cell repellence test of sodium sulfate stabilized, polydopamine patterned, unbaked high-PVA films. Images are taken in phase contrast mode. Left image: successful striped polydopamine patterning and cells adhering accordingly, right image: detached PVA film residue from glass substrate. Scale bar: 100 $\mu$m. Incubation time: 24 h.
4.3 Stabilizing readily soluble PVA thin films

4.3.3 Stabilizing PVA films in mixed borate and sodium sulfate solutions

Stabilizing water soluble PVA films with either borate solutions or sodium sulfate solutions separately did not result in satisfying dissolution properties. Borate solutions did not induce enough cross-linking between PVA chains to stabilize water soluble PVA films. Water soluble PVA films were stable in sodium sulfate solutions but the treatment either did not slow down the film dissolution in water to appropriate rates or made the films completely insoluble. We therefore attempted to mix borate and sodium sulfate solutions to see if the sulfate could stabilize the thin film long enough for the borate to induce cross-linking between PVA chains.

For these tests, we used high-PVA (5%). First a mixed solution consisting of 0.5 M sodium sulfate and 5 mM borate was made at pH 8.8. Surprisingly, neither of the samples exposed in this mixed solution for 10 minutes or 1 hour were dissolution resistant (figure 4.30). All the thin films were completely dissolved after 6 minutes in water.

It was then attempted to increase the pH of this mixed solution to 10.2 to see whether it was possible to activate the PVA cross linking compounds of borate [79]. An exposure time of 10 minutes did not yield any stable PVA films (figure 4.31).

Next, we wanted to see whether higher concentrations of borate would be able stabilize PVA films. We already demonstrated in figure 4.23 that a 50 mM borate solution at pH 10 was able to induce formations of stable hydrogels within seconds. Therefore a mixed solution of 0.5 M sodium sulfate and 50 mM borate at pH 9.9 was prepared. However the samples in figure 4.32 show that both 1 hour and 13 hours long exposures in this mixed solution did not stabilize PVA films, they simply dissolved in water within a few minutes.

![Figure 4.30: Solubility tests of unbaked high-PVA films stabilized in a mixed solution of 0.5 M sodium sulfate and 5 mM borate at pH 8.8. Color fill legend: black: water exposure time, grey: mixed solution exposure time. Unbaked high-PVA films were not stabilized by this solution. Complete dissolution occurred after a few minutes.](image)
Chapter 4. Results  

4.3 Stabilizing readily soluble PVA thin films

**Figure 4.31:** Solubility tests of unbaked high-PVA thin films stabilized in a mixed solution of 0.5 M sodium sulfate and 5 mM borate at pH 10.2. Color fill legend: black: water exposure time, grey: mixed solution exposure time. Unbaked high-PVA films were not stabilized by this solution. Complete dissolution occurred after a few minutes.

**Figure 4.32:** Solubility tests of unbaked high-PVA films stabilized in a mixed solution of 0.5 M sodium sulfate and 5 mM borate at pH 9.9. Color fill legend: black: water exposure time, grey: mixed solution exposure time. Unbaked high-PVA films were not stabilized by this solution. Complete dissolution occurred after a few minutes.
4.4 Destabilizing insoluble PVA thin films with poly(acrylic acid)

Since stabilizing readily soluble PVA films did not give very promising results, we finally attempted to see whether it was possible to destabilize normally dissolution resistant PVA films. Pure PAA films were shown to be water soluble (see figure 4.33) so we mixed equal amounts of high-PVA solutions (5%) with poly(acrylic acid) (5%) solutions and hoped that the these films might be less dissolution resistant than baked high-PVA films. Solubility tests were done in ambient conditions according to the methods described in section 3.1.2 on page 33. The results of the first solubility tests with baked high-PVA:PAA films (figure 4.34) were surprising. The films appeared to be stable after water exposure times as long as 1 hour. The films were even resistant to hot water treatment. We suspected that the PAA solution might be very acidic, inducing an esterification reaction between the carboxylic acid group of PAA and the hydroxyl group of PVA, cross-linking the two polymer types and making the film insoluble. So we prepared new high-PVA:PAA solutions with increased pH by NaOH-adjustment. The results of the solubility tests on the resulting films yielded different interference patterns of a very different color. The color reminded of previous solubility tests in cell medium or of salt deposits from borate solutions. The interpretation of these experiments was very uncertain, but the solubility of baked high-PVA:PAA films was clearly pH dependent.

We also did solubility tests for mixed low-PVA:PAA films (relative concentration 1:1) as well. When first mixing the low-PVA and PAA solutions a white precipitate appeared. Adding a few droplets of 1 M NaOH made the solution colorless again. The results of the solubility tests for the low-PVA:PAA films (figure 4.36) indicated that the films dissolved within 1 minute of water exposure.

These tests were done towards the very end of the project and there was no time to follow up with further experiments.
Chapter 4. Results 4.4 Destabilizing insoluble PVA thin films with poly(acrylic acid)

Figure 4.33: Demonstration of water solubility of PAA-films. PAA concentration: 5%. Water exposure time: 1 min. Images are from before and after water exposure. Within each subfigure – left: unbaked sample, right: baked sample.

Figure 4.34: Solubility test of mixed high-PVA:PAA films (relative concentration 1:1). Images are from before (top) and after (middle) room temperature water exposure (exposure times indicated above the images), and after 20 min hot water rinse (bottom).
4.4 Destabilizing insoluble PVA thin films with poly(acrylic acid)  Chapter 4. Results

Figure 4.35: Solubility test of pH increased mixed high-PVA:PAA films (relative concentration 1:1). Images are from before and after water exposure. Water exposure times are indicated above the images.

Figure 4.36: Solubility test of pH increased mixed low-PVA:PAA films (relative concentration 1:1). Images are from before and after water exposure (1 min).
Chapter 5

Discussion

5.1 Dissolution investigation method review

To perform solubility tests of PVA films, we needed a method for knowing whether the films had dissolved. We previously had developed a method using Si-wafers as substrates for PVA films [1]. When films were present on the wafer surface, clear interference patterns could be seen. By interpreting the interference pattern colors on the samples during the solubility tests we could assess the dissolution properties of the films. In this section, we stress the importance of conducting control experiments when doing solubility tests with wafers, so film solubility can be correctly assessed. In section 4.2.1, solubility tests were misinterpreted. After solubility tests of water soluble low-PVA films in cell medium, remaining interference patterns were observed (see table 4.1). These interference patterns were interpreted as a thinner remaining PVA film on the wafer surface. We later realized that this was a misinterpretation: a simple control experiment showed that the same interference patterns would occur after a blank, PVA free wafers incubated in cell medium for 20 seconds displayed the same interference patterns. These similar interference patterns, combined with the fact we were never able to show the cell repellence of low-PVA films, made us understand that the observed interference patterns were cell medium solute that deposited on the wafer surface during blow drying of the sample. This could be the reason for the difference in measured film thickness using the reflectometer compared to the profilometer. Reflectometer measurements were mostly bigger than profilometer measurements. Since the reflectometer was set to use PVA’s refractive index in film thickness calculations, which is probably not the same as the one of cell medium solute, the reflectometer measurements might be wrong. But we did not pay much attention to this difference as we were primarily interested in the presence or absence of films on the sample surface and the actual film thickness therefore only being of little importance. In the project preceding this one in which the solubility test on wafers and the following dissolution investigation method was developed, all solubility tests were done in water. Solute deposition was therefore never an issue. So when the solubility tests were expanded to be conducted in cell medium during this project it was simply never considered performing a
5.2 Controlled vs. timed dissolution

Chapter 5. Discussion

control experiment for establishing the interference pattern contribution of the solute. For the new solubility tests in section 4.3 involving exposure of PVA films of various solutions, measures were taken to avoid similar misinterpretations. Before each solution exposure we removed some PVA on the corner of a sample so it would become blank (see figure 3.7). If the solute of the solution stabilizing PVA would deposit on the sample surface, it should deposit more or less evenly across the entire sample. In that case, if the solute caused interference patterns to appear on the wafer sample, the interference patterns should appear also on the PVA free corner, and the solute contribution to interference patterns should be easy to detect. Solubility tests using Si-wafers is still a good and simple way of assessing the presence of thin films on the sample surface as long as all other possible sources contributing to creating interference patterns are taken into account, and we can thus be certain that the observed interference patterns are in fact due to PVA films.

5.2 Controlled vs. timed dissolution

The goal of this project was to prepare PVA films in such a way that we could achieve timed dissolution or controlled dissolution of PVA films. The initial idea was to achieve timed dissolution (illustration on figure 5.1a), where PVA films are not completely dissolved until after a few hours, and cells have time to attach on the intended cell adhering surfaces first. From then, a second type of cells can be seeded and grow in the previously PVA covered areas to make patterned co-cultures. The first solubility tests from section 4.2 suggested that certain PVA films were not completely soluble in cell medium but were so in PBS. From this came the idea that instead of timing the dissolution of PVA films, we could simply decide when the PVA would dissolve using controlled dissolution (illustration on figure 5.1b). This would be done by first seeding cells in cell medium and letting them attach to the designated cell adherent areas. Once these cells had attached, cell medium could be removed and PBS added. The PBS would dissolve the PVA quickly, and the PBS could be removed and replaced with new cell medium and patterned co-cultures could be made by seeding new cell types. It turned out that these initial solubility tests in cell medium were misleading, and that controlling PVA dissolution using cell medium and PBS in the way we had hoped was not possible. The concept of controlled dissolution, however, might still be a better idea than timed dissolution. This project has revealed that slowing down the dissolution rates of PVA thin films is very difficult. PVA films either dissolve very quickly or are quickly permanently stabilized. With borate and sodium sulfate treatments we managed to slow down the time needed for complete dissolution or for permanent stabilization from a few seconds to a few minutes. This is not nearly good enough however, as ideally timed dissolution should ideally take a few hours. Furthermore, if very slow dissolution rates would occur and we take into account the thickness of the PVA films, the statistical variation of the amounts of time needed for complete dissolution to occur could be big. If the moment of PVA dissolution could be decided externally and dissolution time could be short, the uncertainty of the complete PVA dissolution would be much smaller. A cell array system with controlled dissolution of the cell repellent PVA layer would be much easier to optimize than a system using timed dissolution. We investigated the possibility of using borate stabilized PVA and triggering PVA dissolution by adding glucose. Borate induces cross-links between PVA chains, and glucose has been
reported to compete with PVA for binding of borate. We therefore hoped that when adding glucose to a system with borate stabilized PVA films, the glucose would bind the borate so that the PVA would no longer be cross-linked, and the PVA would therefore dissolve. The tests for stabilizing PVA with borate were unsuccessful, but should we achieve this in the future, there would still be challenges with the system. Glucose is usually an ingredient in cell medium (we used cell medium with 22 mM glucose), so the glucose concentration in cell medium might have to be altered. Cells will not only have to survive, but also be able to adhere to the polydopamine patterns in a glucose limited, or even glucose free, medium. As cell adherence requires protein synthesis, and glucose is an important source of energy for protein synthesis, this might be problematic. Should it be possible for cells to attach in these conditions, glucose can subsequently be added and all PVA should be dissolved. Then there would no longer be need of keeping the glucose concentration down, and all cells would be free to grow in optimal conditions.

![Diagram](image)

**Figure 5.1:** Conceptual illustrations of timed and controlled dissolution of PVA. The lines from top to bottom are showed in chronological order. The cell adhesive polydopamine layer was not included for simplicity reasons.

### 5.3 Thin films vs. bulk gels

We showed in section 4.3.1 that it was possible to make PVA hydrogels by mixing the borate and PVA solutions. PVA films from the same solutions were not stabilized by exposure to similar borate solutions, however. Borate exposure of baked low-PVA and low2-PVA films appeared to slow down the complete dissolution to a few minutes instead of a few seconds, which was the time needed for non-borate exposed films to dissolve. But the films did not seem to dissolve in the borate solution like unbaked high-PVA films did. The reason for this is unclear. Unbaked high-PVA films should have available hydroxyl groups for borate to interact with, while most of the hydroxyl groups in the baked low-PVA and low2-PVA films should already have formed hydrogen bonds between each other during the baking. Since borate supposedly interacts with the hydroxyl group of PVA, this is surprising. One explanation could be that the low-PVA and low2-PVA films were
thicker than the high-PVA films. The thinner films would simply start dissolving in the water solvent before the borate could have time to interact with the PVA, but slightly thicker films would have time to be stabilized. The larger thicknesses of the low-PVA and low2-PVA films were measured in the work preceding work this project [1]. It can also be expected from their larger molecular weight (see table 3.1 on page 36), as heavier and longer chains would increase the viscosity of the PVA solutions and thereby result in thicker films after spin coating.

5.4 Possibilities for using sodium sulfate to stabilize PVA films

Long exposures of 1 M sodium sulfate (over 30 minutes) was the only alternative method to baking we found for stabilizing PVA films. The problem with this method is that resulting films did not appear to have very different dissolution properties than films stabilized by baking. Sulfate stabilized films were compatible with microcontact printing of polydopamine, so should we be able to find a sulfate treatment inducing the desired PVA dissolution properties, the outlooks would be good. Shorter sulfate treatments (25 minutes and less) did appear to sometimes slow down the dissolution of PVA to a few minutes, but this was not consistent. Other than this minor improvement there was no indication that sulfate exposure was a promising approach to achieve timed dissolution of PVA. Since the sulfate treatment induces hydrogen bonds between PVA chains, like baking, there is no apparent way of achieving controlled dissolution of sulfate stabilized PVA films. It is also worth mentioning that we experienced some problems with adhesion to glass for sulfate stabilized films, but this might only be a matter of optimization. We did not explore different concentrations for the sulfate. However, we did try a 0.5 M sulfate solution mixed with low concentration borate but even long exposure times for this mixed solution (up to 13 hours) resulted in readily soluble PVA films. This, combined with the fact that low concentrated borate solutions had no stabilizing effect on PVA, can lead us to expect that 0.5 M sulfate solutions will not have good PVA stabilization effects.

5.5 Future prospects

During this project we did not achieve the desired dissolution properties of PVA films. Although we tried many different treatments, some parameters should be more thoroughly investigated. For the borate stabilization, we could look into the effect of changing the pH and the concentration one at a time. Since both the pH and the concentration of borate have been reported to have an effect on borate/PVA interactions [78], it would be interesting to see whether specific combinations of these are better at stabilizing PVA than the ones attempted so far. We previously argued that too thin PVA films might dissolve before borate has time to interact with PVA and stabilize it. It could be interesting to increase the film thickness of given film types and see if the PVA film thickness is a decisive parameter. There are apparent challenges with increasing film thickness, however. One possibility could be to spin coat several PVA layers on top of each other. The problem would be that when a PVA solution is pipetted onto the sample prior to spin coating, this solution would
dissolve away underlying PVA films. The only way to prevent this would be to stabilize the PVA films between each time a new layer is spin coated. But the methods we have for stabilizing PVA so far (baking or long sodium sulfate treating) all make PVA films insoluble, so the thickness of the "layer by layer"-PVA film would then be of no importance. Another possibility for increasing the PVA film thickness before stabilizing it could be to increase the PVA concentration of the initial stock solution. Still, there might be a few limitations. Increasing the PVA concentration will eventually saturate the solution. Spin coated films from saturated PVA solutions will be the thickest possible single PVA layer. Also, more concentrated PVA films are more viscous and could result in uneven films after spin coating. The interference patterns of these uneven films would consist of a multitude of colors. Interpreting these interference patterns during solubility tests could be difficult. It is also worth mentioning that thicker PVA films have previously been experienced to have poor adhesion properties to glass substrates [15], so even if we can create optimal dissolution properties for thicker films, the system might not be compatible with a cell array.

The ideal scenario for this project would be to find a chemical stabilization of PVA which is reversible. This way we could apply the principle of controlled dissolution of PVA films. The stimulating compound for reversing the stabilization of PVA, and its bi-products should of course be cell compatible. Finding an appropriate chemical stabilizer is not obvious and we may have to try many different compounds. Should such a compound not be found, we might have to go back to the original approach of the cell array system. The original approach was to coat the cell repellent areas with dopamine and then to induce a polymerization reaction which would result in cell adherent polydopamine coating of the previously cell repellent surfaces. The problem with this system was that the polymerization had toxic bi-products (reactive oxygen species). The strategy then would be to find a way to limit the effect of these.
Chapter 6

Conclusion

In this project, we attempted to achieve timed and controlled dissolution of PVA thin films for application of PVA as a cell repellent layer in a recently developed patterned cell micro-array. PVA film dissolution properties were tested in water and in cell compatible media using Si-wafers as substrates and interpreting the interference patterns of the films. The solubility of PVA films did not appear to be different in water or in cell compatible media. Some solutions did deposit solute onto wafer sample surfaces inducing interference patterns that could be confused with the presence of PVA films. With control experiments we were able to tell PVA induced interference patterns and solute induced interference patterns apart. Tuning of PVA film dissolution properties proved to be difficult. Therefore, several different strategies were investigated. By exposing the films to borate or sodium sulfate solutions instead of stabilizing them through thermal treatment, we were able to slow down the time needed for complete dissolution or permanent stabilization of the films to occur from seconds to minutes. For appropriate timed dissolution, complete dissolution needed to take no less than a few hours, so the dissolution rates we achieved were not satisfactory. We managed to completely stabilize films with long sodium sulfate exposures. However, we did not find a way to achieve controlled dissolution by reversing this stabilization. The cell repellent properties of PVA were not changed by sodium sulfate exposure. We were not able to permanently stabilize PVA films with borate, but we showed that it was possible to make larger PVA hydrogels in borate solutions. Since glucose competes with PVA for binding borate, we hope, in the future, to be able to use glucose to control the dissolution of PVA films. This is very challenging, however. We need better control over the properties of the PVA polymer used. Alternatively, other approaches to make functional cell arrays should be considered.
Chapter 7

Appendix
Figure 7.1: Cell repellence test of low-PVA thin films incubated directly in cell medium with HeLa cells. Control areas were PVA free surfaces on the same low-PVA coated samples as the same sample. Scale bar: 100 μm. Incubation times are noted. The three non-control images are different samples.
Figure 7.2: Cell repellence test of low-PVA thin films incubated once in cell medium which was removed before HeLa cell seeding with new cell medium. Control areas were PVA free surfaces on the same low-PVA coated samples PVA the same sample. Scale bar: 100 µm. Incubation times are noted. The tree non control images are different samples.
Figure 7.3: Cell repellence test of PBS incubated low-PVA films before HeLa cell seeding in cell medium. Control areas were PVA free surfaces on the same low-PVA coated samples PVA the same sample. Scale bar: 100 µm. Incubation times are noted. The three non-control images are different samples.
Figure 7.4: Cell repellence test of low-PVA films incubated in cell medium, then PBS before HeLa cell seeding in cell medium. Control areas were PVA free surfaces on the same low-PVA coated samples PVA the same sample. Scale bar: 100 µm. Incubation times are noted. The tree non control images are different samples.
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