Design of polymeric thin films with nanovolcanoes for trapping hydroxyapatite nanoparticles to promote or inhibit cell proliferation

Maciej Łojkowski (✉ 00183042@pw.edu.pl)  
Faculty of Materials Science and Engineering, Warsaw University of Technology Wołoska 141, 02-507, Warsaw, Poland  
https://orcid.org/0000-0002-0612-7964

Ewa Walejewska  
Faculty of Materials Science and Engineering, Warsaw University of Technology Wołoska 141, 02-507, Warsaw, Poland  
https://orcid.org/0000-0003-2803-4870

Malwina Sosnowska  
Department of Nanobiotechnology, Institute of Biology, The Warsaw University of Life Sciences, Nowoursynowska 166, 02-787, Warsaw, Poland  
https://orcid.org/0000-0002-1882-0846

Agnieszka Opalińska  
Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Sciences Sokolowska 29/37, 01-142 Warsaw, Poland  
https://orcid.org/0000-0002-5210-9257

Kamil Grubczak  
Department of Regenerative Medicine and Immune Regulation, Medical University of Białystok, Waszyngtona 13, 15-269 Białystok, Poland  
https://orcid.org/0000-0002-6828-6397

Sławomir Jaworski  
Department of Nanobiotechnology, Institute of Biology, The Warsaw University of Life Sciences, Nowoursynowska 166, 02-787, Warsaw, Poland  
https://orcid.org/0000-0002-4619-941X

Marcin Moniuszko  
Medical University of Białystok  
https://orcid.org/0000-0001-7183-3120

Wojciech Swieszkowski (✉ wojciech.swieszkowski@pw.edu.pl)  
Faculty of Materials Science and Engineering, Warsaw University of Technology Wołoska 141, 02-507, Warsaw, Poland  
https://orcid.org/0000-0003-4216-9974

Research Article

Keywords: thin polymer films, hydroxyapatite, cell culture, agglomeration, nanovolcanoes, nanoparticle trapping

Posted Date: May 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2868379/v3
License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations:
Figures S1-S3 not available with this version.
Design of polymeric thin films with nanovolcanoes for trapping hydroxyapatite nanoparticles to promote or inhibit cell proliferation

Maciej Łojkowski*, Ewa Walejewska, Malwina Sosnowska, Agnieszka Opalińska, Kamil Grubczak, Sławomir Jaworski, Marcin Moniuszko, Wojciech Swieszkowski*

Maciej Łojkowski* 1, Ewa Walejewska 2, Wojciech Swieszkowski* 8
Faculty of Materials Science and Engineering, Warsaw University of Technology
Wołoska 141, 02-507, Warsaw, Poland
Email: Maciek.lojkowski@gmail.com; wojciech.swieszkowski@pw.edu.pl

Malwina Sosnowska 3, Sławomir Jaworski 6
Department of Nanobiotechnology, Institute of Biology, The Warsaw University of Life Sciences, Nowoursynowska 166, 02-787, Warsaw, Poland

Kamil Grubczak 5 Marcin Moniuszko 7
Department of Regenerative Medicine and Immune Regulation, Medical University of Białystok, Waszyngtona 13, 15-269 Białystok, Poland

Agnieszka Opalińska 4
Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Sciences Sokolowska 29/37, 01-142 Warsaw, Poland.

Keywords: thin polymer films, hydroxyapatite, cell culture, agglomeration, nanovolcanoes, nanoparticle trapping
1. Introduction

Nanotechnology has long been of interest as a tool for research in cell culture. Advances in nanotechnology allow tuning of the adhesive properties of the surface.\[^1\] The focal adhesion pathways control the live cycle of the cells, allowing the cells to form firm adhesion with the environment and proliferate.\[^2\] On the other hand, the chemical properties of substrate properties through mechanotransduction can arrest the cell cycle and inhibit its proliferation.\[^3\] In particular, hydrophobic surfaces can reduce the cell adsorption of molecules, CAMs, while hydrophilic surfaces can promote cell adhesion.\[^4,5\] Moreover, the binding of many adhesion proteins is calcium-dependent.\[^6\] Over 99% of total body calcium is found as calcium hydroxyapatite, which is located in bone and teeth.\[^7\] Thus, its addition to the bone tissue engineering (BTE) field can improve cell adhesion.

Hydroxyapatite can serve as an anchoring point for forming new focal points. Up to this point, hydroxyapatite nanoparticles, HAp, were applied successfully in BTE in polymer and titanium bone implants or dental cements.\[^8–11\] An effective, extensively used method for synthesizing HAp nanoparticles is the solvothermal method, which produces nanoparticles in a microwave reactor. Recently, a method for the large-scale production of hydroxyapatite nanoparticles with precisely controlled size, excellent biocompatibility, and chemical structure that resembles the hydroxyapatite inside the bone was developed.\[^12,13\]

One of the challenges of nanotechnology is to control the material on the nanoscale. For example, to precisely manufacture functional surfaces with designed patterning. Such patterns were successfully designed for chemical and refractive index sensing or precisely controlled protein adsorption.\[^14–18\] Polymer thin films are an effective and versatile strategy in manufacturing various functional devices. Typically, this method involves spin-coating two or three components from a solution. As a result of evaporation of the solvent, liquid-liquid phase separation occurs. In this way, a micropattern is formed, which can be used to prepare selective binding sites, where one phase can be dissolved to obtain nanocavities.\[^19\] Spin-coating involves placing a drop of solution on a spinning disk.\[^20\] The centrifugal force spreads an even layer of liquid over the surface. This allows rapid evaporation of the solvent. As a result of this process, high-speed production is possible, and ease and flexibility of the process followed by relatively high-cost efficiency is an advantage.\[^21\] One of the effects of microphase separation is the occurrence of surface roughness.\[^22\] The roughness also plays a crucial role in cell adhesion.\[^23\] The roughness increases the surface area available for the binding sites. The roughness is also linked to the wettability of the surface expressed by the wetting contact angle CA. A higher surface area increases the surface free energy and, thus,
wettability. However, if the surface is hydrophobic, the increased surface area would decrease the wettability and increase the wetting angle. This wetting regime is called the Wenzel state.\[^{[24]}\] On the other hand, if the steepness of the roughness increased enough, the wetting could transform into the so-called Cassie-Baxter regime.\[^{[25,26]}\] The cavities in the Cassie-Baxter state are filled with underwater bubbles, which decreases the surface energy and can result in a superhydrophobic surface. Two examples of such surfaces that were also found to reduce biofilm formation are lotus leaf-like surfaces consisting of an array of sharp spikes or cicada wing-like surfaces with a close-packed array of submicron holes.\[^{[27,28]}\]

The array of holes, as mentioned above, can be obtained by utilizing the film with microphase separation and subsequently dissolving the phase that forms islands. Another strategy utilizes breath figure formation. Breath figures, BFs, occur when air humidity condenses on the surface of the liquid solution during spin-coating. It is associated with forming an array of micron-scale droplets that sink into not yet solidified film. After the water droplets evaporate, an array of holes appear.\[^{[29]}\]

The phase separation between two incompatible polymers is well understood. However, little information exists about the residual structures left after the selective lift-off of the phase that forms the islands. These structures look like small nanoscale volcanoes, NVs. The specific shape of these structures can have an interesting effect on surface wettability due to their aerophilicity.\[^{[30]}\]

Here, we seek to design a surface that would enable the capture of nanoparticles from an aqueous suspension. For this purpose, we chose to work with the HAp nanoparticles. The unique shape of the nanostructures, externally resembling volcanoes but with a concave hollow interior, allows the air bubble to be enclosed inside the structure. The apex of the volcano is where the air-water interface is pinned. Such an aerophilic surface can be used to trap nanoparticles by rapid release of air.\[^{[31]}\] A bubble escapes from the nanostructure when the pressure in the sample's chamber is lowered. Following the escape of the bubble inside the nanostructure, a subpressure is created, and the nanoparticle suspension is aspirated into the crater. Up to this point, subpressure aspiration of nanoparticles into nanovolcanoes was demonstrated to trap 50 nm silica nanoparticles using light-driven colloidal nanolithography.\[^{[31]}\] However, in our research, we first demonstrate the aspiration of nanoparticles into nanovolcanoes prepared with spin-coating methods. Moreover, to our knowledge, this research is the first to demonstrate the aspiration of hydroxyapatite particles and the adhesion/nonadhesion properties of such surfaces, depending on whether the HAp is
trapped inside. Thus, a simple method was developed to prepare adhesive and nonadhesive surfaces for cell culture.

To achieve the best possible efficiency of trapping nanoparticles into the interior of nanostructures, it is essential to obtain an adequate geometry of these nanostructures. We investigated how the shape of the polystyrene island, PS, formed during the microphase separation between polystyrene and poly(methyl methacrylate), PMMA. The films of the PMMA/PS blend were prepared via spin-coating. The PS islands were intended to perform as a mold for the NVs, inheriting the silhouette from the dissolved polystyrene islands. We proposed a system based on PS with a bimodal molecular weight distribution, bi-MWD, to address this issue. Such polystyrene consists of two types of chains, one with a lower molecular weight and the other with a higher molecular weight. Multiple studies have shown the migration of lower molecular weight components toward the interphase region to lower the system's energy.\textsuperscript{[32,33,33,34]} The higher molecular weight component tends to stick to the bulk. The third component is an intermediate phase between the film matrix and the islands.\textsuperscript{[22,35–37]}

We have recently shown how polystyrene consisting of two dominant molecular weights in a bimodal molecular weight distribution can be separated by vapor condensation to form a bimodal morphology in a thin film.\textsuperscript{[38]} Furthermore, we found that the separation in polystyrene was related to the strength of polar interactions and hydrogen bonds between the solvent and polystyrene, i.e., MEK. i.e., methyl ethyl ketone, MEK is a better solvent for 20 kDa polystyrene than for 200 kDa polystyrene.\textsuperscript{[39]} We also showed how to describe the shape of nanovolcanoes with an autocorrelation function and that the so-defined steepness parameter is related to surface wettability.\textsuperscript{[30]}

In the current paper, we investigated how to tune the nanovolcano shape using PS islands as a mold and changing the shape by modifying the PS molecular weight distribution and precisely controlling the humidity during spin-coating. The ability of nanovolcanoes to store air bubbles was tested. The nanovolcanoes filled with hydroxyapatite, NVs@HAp, and the NVs without hydroxyapatite were tested in cell culture for their biocompatibility. The composite surface, NVs@HAp, positively affected cell proliferation and spreading. In contrast, pristine NVs inhibited cell adhesion and proliferation. The demonstrated method can be further enhanced by trapping different functional nanoparticles and/or other micropatterning techniques to produce novel functional devices.
2. Results and discussion

2.1 Designing nanovolcanoes using island modifications to change their shape

![Diagram of fabrication process]

**Figure 1.** A – Fabrication of thin film from a solution of PMMA and PS with bimodal MWD. B – the lighter element migrates toward the surface, changing the island's shape. C – the island is dissolved with a selective solvent. D – By controlling the MWD of the PS and the concentration of the solution, the desired shape of the islands can be designed.

To obtain nanovolcanoes, NVs with different geometries to enable HAp trapping in later stages, polystyrene islands, PS, formed by microphase separation, were used as molds to tune the NVS shape. A method was developed to change the shape of the islands by using a blend of PS with two different molecular weights. As a result of the difference in solubility of PS in methyl ethyl ketone (MEK), the islands change shape as a function of humidity level during spin-coating and as a function of mixture composition.

The idea is presented in **Figure 1.** A solution of PMMA and PS with bimodal MWD was spin-coated, resulting in PS island formation due to phase separation. A selective solvent is used to dissolve the PS phase. Typically, a very fine ring of residual matter can be found surrounding the hole that remained after the islands were lifted off. We have found that these residuals’ heights can be tuned. The structure that forms resembles nanoscale volcanoes. In particular, humidity during spin-coating is essential for the growth of nanovolcano slopes.

Changing the bimodal MWD of PS islands, the desired geometry of the NVs can be obtained. PS with a low Mw migrates toward the interface, changing the interfacial region's surface energy and solubility.
The assumption was made that the NVs can store air bubbles. When the sample decorated with NVs is submerged in the NP suspension and placed in a vacuum chamber, the air bubbles escape, and as a result, the formed under pressure causes the aspiration of NPs into the NVs (Figure 2 A)). The residual HAp was then blown away, washed, and dried. However, geometry tuning is necessary to find the right conditions for trapping the NPs inside the NVs. Figure 2 B) shows the projection of the surface with NVs obtained by AFM depicting the NVs without HAp and with HAp trapped inside.

Figure 2. A) Schematic depiction of HAp nanoparticle trapping by the release of air bubbles from the nanovolcanoes. B) 3D projections of the AFM images showing the surface before and after nanoparticle trapping.

2.2 Effect of the humidity during spin-coating and the blend composition on nanovolcanoes' growth

To find the right shape of the NVs for HAp trapping, several blends (Table 1) were dissolved in MEK and spin-coated on the SiO substrate. We adopted this naming convention: C - concentration; molecular weights of individual polymers in order: Mw of PMMA, PS\textsubscript{L}, PS\textsubscript{H}; the ratio of PS\textsubscript{L} to PS\textsubscript{H}; humidity during sample fabrication. The blends with PS with bimodal MWD were also compared with PS with uniform MWD. We characterized the shape of the islands, and next, we compared them to the shape of NVs to understand how the characteristics of the islands affect the slope of the nanovolcano. The AFM images of the blends are shown in Fig. S1, Supporting Information, SI, followed by the power spectra.
density, PSDF, analysis of the morphology change with respect to the blend composition and relative humidity, Rh (Fig. S2, SI).

**Table 1.** Summary of polymer blends’ molecular weight used in this research; composition code should be read as: Mw of PMMA, PS_L, PS_H; ratio PS_L to PS_H.

<table>
<thead>
<tr>
<th></th>
<th>2009200; 2575</th>
<th>2009200; 5050</th>
<th>2009200; 7525</th>
<th>2020200; 2575</th>
<th>2020200; 5050</th>
<th>2020200; 7525</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn [kDa]^a</td>
<td>173</td>
<td>146</td>
<td>118</td>
<td>155</td>
<td>110</td>
<td>65</td>
</tr>
<tr>
<td>Mw [kDa]^a</td>
<td>186</td>
<td>166</td>
<td>137</td>
<td>194</td>
<td>184</td>
<td>158</td>
</tr>
<tr>
<td>PDI^b</td>
<td>1.08</td>
<td>1.14</td>
<td>1.16</td>
<td>1.25</td>
<td>1.67</td>
<td>2.43</td>
</tr>
</tbody>
</table>

^a) \( M_\text{w} = (f_1 M_\text{w1} + f_2 M_\text{w2})/(f_1 + f_2), M_\text{n} = f_1 M_\text{n1} + f_2 M_\text{n2}, f - \text{fraction of one of the polymers in } \%; \)

^b) \( \text{PDI} = M_\text{w}/M_\text{n} - \text{polydispersity index.} \)

When the NVs were prepared using PS with a uniform MWD, they tended to crack when submerged and subsequently subjected to vacuum (Fig. S3, SI). It was assumed that they cracked while releasing the air bubbles.

It was also necessary to distinguish a suitable humidity range for sample preparation. Interestingly, breath figures, BFs, appeared on the surfaces at lower humidity levels when PS with uniform Mw was used compared to when bimodal MWD was used.

![Normalized radius of breath figures](image)

**Figure 3.** The normalized radius of breath figures depending on PS MWD and spin-coating humidity. Lighter colors depict the blends with uniform PS, and darker colors are for PS with bimodal MWD.
Figure 3 shows the normalized radius of BFs as a function of spin-coating humidity and blend composition. The BFs were found for a humidity of 35% for the 20 kDa PMMA and 20 kDa PS blend or a humidity between 40% and 50% for blends consisting of PS with Mw between 70 kDa and 200 kDa. For islands with PS with bimodal MWD, BFs were found for humidity higher than 50%.

When 2020200, 7525, or 5050 blends were used, the growth of islands from very small, 25 nm height was possible. The growth of the islands, as depicted in Figure 4 A, was correlated with increased humidity during spin-coating. However, for blends with higher average molecular weights, the spinodal-like morphology formed for humidity below 25%. Thus, the region of interest was between 25% and 50% Rh.

![Figure 3](image)

Figure 4. A - The profiles of the islands for Rh from 0% to 70%. Blend C25;2020200; 5050 was used, and different humidities were applied during spin-coating. B - Cube volume aspect ratio of the islands regarding the blend composition and humidity. C – the average radius of nanovolcanoes, $r_c$, and the average height of nanovolcanoes, $h_c$, with a breakdown by blend type. D - the average ratio of NVs height, $h_c$, to island height, $h_i$, and the ratio of average NVs crater diameter, $r_c$, to average island diameter, $r_i$. 
The composition of the mixture affected the shape of the islands, as shown in Figure 4 B. Values of the cube-volume aspect ratio of islands close to unity indicate that the base and height are similar in value. In contrast, the larger the aspect ratio, the flatter the island appears.

Interestingly, the blends prepared from uniform PS blends were the steepest. At the same time, the blends with bimodal PS were clearly dependent on humidity. They became steeper with the increase in the fabrication humidity, finally matching the steepness of the blends with uniform PS at a humidity of approximately 50%. The lowest values were for a PS molecular weight of 20 kDa. For blends with bimodal molecular weight polystyrene, the order was 2020200 blends were flatter than uniform blends, and the 2090200 blends were flattest.

Selective solvent etching was used to dissolve the PS islands. As a result, hollow cavities were created surrounded by the slopes of NVs. To illustrate the influence of blend composition on the slope height, $h_c$, and radius of the crater at the top of the slope, $r_c$, the average values for the humidity between 25% and 50% are presented in Figure 4 C. The radius of NVs can be tuned by increasing the PS molecular weight. Figure 4 D shows the formation of the NVs. Here, the values for the 2020200 and 2090200 blends were taken together as an average value and compared. Noticeably, the 2020200 blends had lower ratios between the height of islands and the height of NVs and the radius of NVs and the radius of the island than the 2090200 blends. The corresponding values are given in Table 2.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$h_c/h_i$</th>
<th>$r_c/r_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C25; 2020200</td>
<td>0.75 ± 0.07</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>C25; 2090200</td>
<td>0.81 ± 0.03</td>
<td>0.48 ± 0.04</td>
</tr>
</tbody>
</table>

$^a$h$_c$ – the height of nanovolcanoes crowns, $h_i$ – the height of the islands; $^b$r$_c$ - radius of nanovolcanoes peaks, $r_i$ – radius of islands.

The slopes of nanovolcanoes can be adequately tuned due to the migration of the light component toward the interphase volume. The interphase thickness is proportional to the molecular weight $d \propto (M/\chi)^{1/2}$. Hence, it would follow that the area in which the two polymers are mixed is thicker for higher molecular weight polymers than for lighter molecules. However, at the same time, the wider MWD would also result in a thicker interface. It should also be assumed that how high the PMMA-rich phase climbs depends on the surface tension between the two phases, which is also related to the interphase thickness and the molecular weight of the polymers. A larger interfacial width is one characteristic.
of blends with a larger molecular weight distribution. It is possible that when polystyrene is subjected to dissolution, the walls of the nanovolcanoes remain thicker for blends with bimodal molecular weight distributions. In particular, the light molecular weight phase tends to migrate towards the interface, as it is a lower energy component.\textsuperscript{[32,33]}

In terms of Hansen solubility, the distance $R$ can be used to describe the affinity between the polymer and the solvent (eq. 2). The molecular weight or end group chemistry can affect the dispersive, $D$, polar, $P$, components or hydrogen bonds, $H$.\textsuperscript{[44]}

\begin{equation}
R = \sqrt{4(dD_1 - dD_2)^2 + (dP_1 - dP_2)^2 + (dH_1 - dH_2)^2}
\end{equation}

The height of the features that remain after PS etching will also depend on the surface tension and the ability of the PMMA-rich phase to wet the PS islands. The surface tension between the PMMA-rich and PS-rich phases could drive the PMMA phase up the concave PS island through capillary action. Another mechanism could be that the PMMA phase was pinned to the PS island at some height. As the PMMA phase shrunk due to solvent evaporation, the pinned part remained higher than the rest of the PMMA phase. It has also been argued that the relation between the height of islands and the matrix can be due to the higher affinity of the solvent to one of the components. The solvent would flow towards the component, depleting the second one and causing faster solidification. Then, a more mobile phase could be squished upward.\textsuperscript{[16,45]}

On the other hand, PMMA should become depleted from the solvent earlier due to its lower molecular weight. Thus, the positions of the islands in place are fixed. Taking the higher solubility of PMMA in MEK, the solubility difference could, however, play a role in the interfacial region and be responsible for the observed phenomenon when the PMMA forms a convex ring around the islands. The Hansen solubility distance (eq. 2) between PMMA and MEK, $R=5.83$, is lower than that between PS and MEK, $R=6.43$. A recent paper demonstrated that 20 kDa PS has a higher affinity for MEK, $R=5.26$, than 200 kDa PS, $R=6.47$, and PMMA.\textsuperscript{[39]} The solubilities between PS and PMMA and PS and PMMA and MEK, in relation to PS $\text{Mw}$, are shown in Figure S4, SI. Regarding the height difference, the AFM measurements of the profile of the scratched films composed of a single polymer with selected molecular weight allowed us to measure the thickness of the films. These are summarized in Figure S5, SI.

Referring to Figure 4, it is worth noting that while the height of the islands can be related to the polystyrene's molecular weight, the islands' profile and how high the PMMA phase can reach depends on the molecular weight of the lighter PS fraction.
2.3 Statistical characteristic of nanovolcanoes

Figure 5. A - Examples of the profiles of the NVs at a humidity; different PS compositions were used as molds; Samples were prepared at a humidity of 40%. B – Growth of the characteristic length scales $\sigma$, $\xi$, and $\sigma/\xi$ in films with NVs with increasing humidity. $\sigma/\xi$ shows values for the humidity range of 25% to 50% only.

Representative profiles of NVs are depicted in Figure 5 A. Profiles of NVs with similar diameters were used for comparison, but they were fabricated from different blend compositions. As intended, the silhouette of the NVS profile was inherited from the shape of the PS island. The statistical analysis of the AFM images of the films is presented in Figure 5 B. The simple characterization of the film morphology by the surface roughness, $\sigma$, is often insufficient to determine the shape effects of the nanostructures. However, finding the characteristic length scales, for example, the autocorrelation length, ACL, or $\xi$, can provide statistical information about the average geometry of the structures. $\sigma/\xi$ allows us to determine the average undulation of the surface by finding the ratio between the root mean square roughness and the characteristic length of the structures. Such analysis allows us to compare the steepness of nanoscale features regardless of the length scale.

We found that the blends with composition 2020200 had higher $\sigma/\xi$ values than the 2090200 blends, and the 2575 PS$_L$ to PS$_H$ blends had lower $\sigma/\xi$ values than the 7525 and 5050 blends. When uniform PS was used for NVs formation, the $\sigma/\xi$ parameter was subjected to sudden changes. This can be related to the transformation between the bicontinuous morphology formed due to spinodal decomposition and the islands' morphology at low humidity. At higher humidity, the changes should be linked to BF formation. In contrast, steady growth in relation to the humidity increase was found when the bimodal PS MWD was utilized. Furthermore,
features were shifted towards the left towards shorter autocorrelation lengths for the blends with the composition of 2020200, signaling higher undulation.

2.4 Dependence of wettability on nanovolcanoes geometry

The connection between surface roughness and the surface wettability has long been utilized to produce hydrophobic surfaces. The steepness of the nanostructures can induce the hydrophobic effect due to air being captured in the cavities.[26,51] This is also often called the Cassie-Baxter law. It has also been proven that the pores, which are concave inside, can store air better. Such surfaces were utilized to produce hydrophobic and oil-phobic materials. In the case of such shaped structures, the air-liquid interface tends to be pinned to the sharp pore edge.[52,53]

![Figure 6](image)

**Figure 6.** Wettability analysis. A – Change in contact angle before and after PS etching for a set of samples manufactured at a humidity of 40%. B – Projected contact angle dependency on the $\sigma/\xi$ parameter (eq. 4a and 4b). C, D – Relation between the contact angle and humidity of the spin-coating process Rh%.

When the PS phase is etched off, the water contact angle (water CA) of the samples significantly increases. We investigated the average contact angle for all kinds of samples manufactured at a humidity of 40% and 25 mg/ml concentration (Figure 6 A). It was found that the CA had only slight deviations across the entire set. The measured CA before etching was $76^\circ \pm 2^\circ$, while after etching, it was $103^\circ \pm 3^\circ$. PMMA is a hydrophilic polymer with a typical contact angle of $73^\circ$. The measured value was slightly higher. The CA of the two-component blend can be found using the Cassie-Baxter equation, C-B (eq. 3).

$$\cos\theta^* = \Delta S \phi_s \left(\frac{y_s - y_f}{y_f}\right) - (1 - \phi_s) - \frac{\gamma_{\text{strain}}}{y_f}$$ (3)
Here, $\theta$ is the wetting contact angle, $\Delta S$ is an excess surface area due to the surface roughness, $\gamma_{sa}, \gamma_{sl}, \gamma_l$ is the surface tension: $sa$ – surface – air, $sl$ – surface liquid, $l$ – liquid-air; $\phi_{a}$ - an area of air trapped between the liquid and surface. The typical CA of PS is $\sim 84^\circ$, and the average PS fraction measured from AFM images was $26\% \pm 8\%$. For these assumptions, $\text{CA}_\text{C-B} \sim 76^\circ \pm 3^\circ$, with excellent agreement with the experimental data.

As PMMA is a hydrophilic polymer, the increase in surface area due to etching cannot account for the increase in CA to $103^\circ \pm 3^\circ$. However, this value can be explained in terms of the Cassie-Baxter equation, with the PS phase being replaced by air bubbles trapped inside the cavities. However, under the assumption that the surface is composed purely of PMMA and the air surface fraction is $\sim 10\%$ on average, as measured by AFM, the C-B equation yields $\text{CA}_{\text{C-B, Air}} = 80^\circ$, considerably less than the measured contact angle, assuming $\gamma_{\text{strain}} = 0$. One of the explanations can be the nanoscale curvature of the surface, causing increased pressure inside the cavities and the undulation of the wetting contact line, resulting in nonzero $\gamma_{\text{strain}}$.

The pressure and curvature are related by Laplace Law, $\Delta p = -2\gamma_l\kappa$, where $\gamma_l$ is the surface tension of water and $\kappa$ is the curvature.

In ref.$^{[30]}$, we investigated surfaces with the same architecture, where the distribution of spots was maintained the same, but the height of NVS was changed. Thus, only the slopes were varied, while other parameters were kept constant. It was proposed that the water contact angle can be related to $\sigma/\xi$, assuming the correction for the C-B equation in the form of eq. 4a for advancing contact angle, ADV, and eq. 4b for a receding contact angle REC$^{[30]}$.

\[
\cos \theta^*_{\text{ADV}} = \phi_{\text{PMMA}} \cos \theta_{\text{PMMA}} - \phi_{\text{AIR}} - \frac{0.1}{\gamma_{H_2O}} \left( \frac{\sigma}{\xi} \right) + 0.05 \quad (4a)
\]

\[
\cos \theta^*_{\text{REC}} = \phi_{\text{PMMA}} \cos \theta_{\text{PMMA}} - \phi_{\text{AIR}} - \frac{0.13}{\gamma_{H_2O}} \left( \frac{\sigma}{\xi} \right) + 0.6 \quad (4b)
\]

Taking some actual $\sigma/\xi$ values, for example, $48/247 = 0.19$ or $35/135 = 0.26$, the corresponding advancing contact angles are $93^\circ$ and $98^\circ$, and the receding contact angles are $65^\circ$ and $72^\circ$, respectively. The measured advancing CAs were $\sim 102^\circ$ and $106^\circ$, and the receding CAs were $\sim 68^\circ$ and $74^\circ$, respectively. This relation is not as straightforward here, as more variables are subjected to a change, such as the density and diameter of air bubbles. The projected relationship between the CA and $\sigma/\xi$ using equations 6a and 6b is shown in Figure 6 B.

Figures 6 C and D illustrate the change in contact angle with the manufacturing humidity on an example of 2020200 compositions. When the spin-coating humidity was less than 25%, the PSI to PSH ratio strongly influenced the CA. For Rh% between 30% and 50%, the CA is close
to approximately 100°. The CA slightly increases for the 75:25 composition and decreases for the 25:75 composition. For Rh% above 50%, the contact angle intensively increased. It is correlated with the BFs that formed on the surface and increased the porosity.

For slope coefficients between 0.3 and 0.4, the droplet could slide off the surface with low hysteresis. For example, in Figure S6, SI, the measurement of the base diameter of the droplet and the contact angle when the droplet is left to evaporate freely is presented. The contact angle was stable at ~89° for the time range between 100 seconds and 350 seconds, while the diameter of the droplet decreased due to evaporation.

2.5 Tuning the capability of NVs to store air bubbles

To optimize the trapping of the nanoparticles, we have investigated whether the NVs can store air bubbles and for how long it is done. In-liquid AFM working in contact mode proved to be an effective method for imaging submerged air bubbles.[54] The samples were submerged in deionized water, and one chosen spot was scanned three to four times to investigate how long the bubbles would stay inside the NVs.

The air bubbles tended to disappear during scanning when they were prepared at 25% or 50% humidity while being stable when samples prepared at 40% or 60% humidity were investigated. As the bubbles faded away, the air-liquid interface descended lower into the nanovolcano interior. In Figure 7, three representative profiles of NVs prepared at humidities of 25%, 40%, and 50% are presented alongside the corresponding images of the samples with air bubbles trapped inside the NVs. The most stable air bubbles were those that were pinned to the top of the nanovolcano. More sample images spin-coated at a humidity of 40% with trapped air bubbles are shared in Figure S7, SI. The condition for stable air bubbles was \( \sigma/\xi \) at least 0.24. The related values describing the geometry when the air bubbles were most stable are summarized in Table 2.
The scale bar is 2 μm, dotted black lines depict the profile of the air bubble, and solid lines depict the profiles of the nanovolcanoes without air bubbles. The profiles were chosen to match in diameter.

Table 2. The characteristics of NVs with air bubbles during AFM imaging. Selected samples were prepared at Rh 40%.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>σ/ξ</th>
<th>r [nm]</th>
<th>h [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C35; 2090200; 50/50</td>
<td>0.28 ± 0.07</td>
<td>191 ± 92</td>
<td>180 ± 32</td>
</tr>
<tr>
<td>C35; 2020200; 75/25</td>
<td>0.3 ± 0.05</td>
<td>233 ± 46</td>
<td>202 ± 23</td>
</tr>
<tr>
<td>C35; 2020200; 50/50</td>
<td>0.24 ± 0.06</td>
<td>300 ± 80</td>
<td>211 ± 42</td>
</tr>
<tr>
<td>C25; 2020200; 75/25</td>
<td>0.26 ± 0.04</td>
<td>220 ± 63</td>
<td>85 ± 20</td>
</tr>
<tr>
<td>C25; 2020200; 50/50</td>
<td>0.18 ± 0.05</td>
<td>213 ± 61</td>
<td>106 ± 25</td>
</tr>
<tr>
<td>C25; 2090200; 50/50</td>
<td>0.17 ± 0.03</td>
<td>154 ± 61</td>
<td>95 ± 20</td>
</tr>
</tbody>
</table>

a) Bubbles were escaping during the first 10 min of scanning; b) Bubbles were present for at least 30 min; r – mean radius of NVs openings; h – mean height above the matrix.

2.6 Trapping HAp inside nanovolcanoes

On the basis of the aforementioned investigation, the sample composition was chosen for the HAp trapping experiments. In the designed system, the surface was easy to rinse off excess nanoparticles. Although later surface modifications can make cleaning the surface more effective, in this phase of testing, we decided to see if the mere hydrophobicity of the surface
decorated with nanoparticles could contribute to the effective removal of excess nanoparticles. The HAp trapped in NVs is depicted in Figure 8. The representative images of the samples depict the successful trapping of HAp nanoparticles inside nanovolcanoes. However, it was found that when the surface was too hydrophobic, i.e., C25; 2020200; 75/25; made at Rh 40%, the excess nanoparticles were easily rinsed off, but particle entrapment did not occur either. This could have happened if the air bubbles were firmly pinned inside NV. The surface prepared with the same composition but made at a lower humidity, RH 35%, than HAp was found inside the cavities. Similarly, the NVs made with blend C25; 2090200; 50/50 prepared at Rh 40% were empty as well. However, this time, the washing of HAp was hindered. Samples prepared at 35% were more suitable to trap HAp nanoparticles. The formation of BFs, i.e., at a humidity of 60%, made the increase in excess nanoparticles impossible. As in the air-trapping investigation, larger pores were less likely to be filled with nanoparticles. An interesting HAp trapping sample offering a smaller diameter NVs set was prepared from a mixture of C25; 2020200; 90:10; 6:4; at Rh 45%. Considering all samples, the mean diameter of cavities with HAp was 344 nm ± 102 nm, and $\sigma/\xi$ was 0.29 ± 0.08.

![Figure 8](image.png)

**Figure 8.** AFM images of the representative set of samples demonstrating HAp trapping. The image size was 10 μm x 10 μm.

The trapping of HAp nanoparticles was found to be the most effective when an aged suspension was used. The suspension was left to settle down to form a close-to-equilibrium
composition. It usually took approximately 3 h for the solution to settle down. The partition of the HAp suspension is depicted in Figure 9 A). According to the moisture analyzer, the sedimented fraction consisted of 34% HAp. The supplied BET characteristics of the nanoparticles showed that their average size was 19 nm. The DLS characterization of nanoparticles in suspension showed average dimensions of agglomerates of 743 nm and 1461 nm for the sedimented fraction and 162 nm and 4766 nm for the suspended fraction (Table 3). Previous SEM analyses indicated that HAp agglomerates were 100 nm - 150 nm in size when deposited on a surface.\cite{12, 55} Thus, it can be assumed that the size of the agglomerates was smaller than the diameter of the NVs. A droplet of a thickened suspension was placed on the sample, and the sample was placed in the vacuum chamber to degas the air bubbles and create the subpressure. The size of the droplet can be adjusted. The hydrophilicity changed after the samples were cleaned if HAp trapping was successful. Water easily slipped from the surface without HAp but was stuck where the HAp was trapped (Figure 9 B), C). As expected, the measured wetting angle on the HAp-modified surfaces decreased to 82° from 102°. Figure 9 D presents the FTIR spectra of samples before and after HAp deposition. The bands associated with HAp are seen at 1052 cm$^{-1}$ and 560 cm$^{-1}$. The related FTIR spectrum of pure HAp is shared in Figure S8, SI.

<table>
<thead>
<tr>
<th>Table 3. DLS analysis of hydroxyapatite agglomerate size in suspension.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1 [nm]</td>
</tr>
<tr>
<td>HAp sedimented fraction</td>
</tr>
<tr>
<td>HAp suspended fraction$^a$</td>
</tr>
</tbody>
</table>

$^a$The missing fraction was assumed to be an artifact.

Figure 9. A) The aged suspension of HAp settled, forming a gel-like fraction on the bottom of the bottle, was pipetted on the sample's surface, and subjected to vacuum. B) Water stuck to the areas where the HAp was successfully trapped inside NVs and easily slipped off the surface without HAp. C) Contact angle with or without HAp. D) FTIR spectra of samples with and without HAp.
2.7 Morphology and metabolic activity of cells on samples with and without trapped hydroxyapatite

![Images of cells on NVs with and without hydroxyapatite after 72 h of culture. A) Scanning electron microscopy and B) confocal images of L929 and MG-63 cells.](image)

Figure 10. Images of cells on NVs with and without hydroxyapatite after 72 h of culture. A) Scanning electron microscopy and B) confocal images of L929 and MG-63 cells.

The bioactivity of samples without HAp (NVs) and with HAp trapped inside nanovolcanoes (NVs@HAp) was demonstrated using samples prepared with composition and humidity of C25; 2090200; 5050, and Rh 35%, respectively. Other compositions, such as C25 2020200; 7525; or C25; 2020200; 6:4 (PMMA to PS); 9:1 (PS to PSi), could also be suitable. The adherent cell lines mouse fibroblasts L929 and MG-63 human osteosarcoma were used. The bottom of the polystyrene well plate was used as a control. We have decided to use the bone-originating MG-63 cell line, as it is widely used for testing the biocompatibility of bone cement and implants. Therefore, cells should be sensitive to the presence of HAp. The cell
concentration was chosen to stop the experiment before cells formed a monolayer. Their viability was assessed after 72 h.
Sporadic, shrunken cells in the case of both cell lines with spherical morphology were found for the NVs without HAp (Figure 10). In contrast, cells cultured on the surface with HAp trapped inside NVs showed an elongated shape characteristic of fibroblast-like cells (mesenchymal phenotype). Higher cell density was observed on NVs coated with HAp compared to NVs.
Shape analysis from confocal microscopy images reiterated these observations (Figure 11 A). The percentage of area covered by cells increased several times, from approximately 1% to 10%. In addition, cell polarity increased significantly, i.e., the length-to-width ratio increased from 1.3 to 1.8 for MG-63 (no HAp vs. HAp addition) (Figure 11 B). Interestingly, the increase in polarity was less pronounced for the L929 cell line. To test cell metabolic activity for NVs enriched with HAp, an XTT viability assay was performed (Figure 11 C). The results showed an absorbance increase of 2.1 for L929 and 2.3 for MG-63 compared to the control. Thus, NVs with HAp was nontoxic for cells and their mitochondria and probably caused an increase in their metabolic activity (or proliferation) and adhesion strength.

![Figure 11](image_url)

**Figure 11.** Effect of nanovolcanoes, NVs with and without (control) hydroxyapatite, HAp, on the morphology and metabolic activity of L929 and MG-63 cells according to image analysis and XTT assay. A) Area of surface in percent of total image area covered with cells after 72 h. B) Polarization of the cells as the ratio of the long axis to the short axis. C) XTT assay of cells on NVs with HAp as a fraction of control activity.

3. Conclusions
We have demonstrated a method for trapping nanoparticles inside nanometer-scale cavities using hydroxyapatite nanoparticles. To this end, we have developed a method to change the geometry of the cavities so that they are able to store air bubbles after the sample is immersed in water. These cavities are formed when the island-forming phase is dissolved with a selective solvent. The islands can be considered a form that modifies the interior of the cavities. Due to the influence of the mass distribution of the particulate phase of the polymer blend that formed the islands through phase separation, the geometry of the islands can be influenced since the lighter component of the blend tends to migrate toward the interphase space, thus measuring the tension on the surface of the islands and the width of the interphase width.

Combined with the change in humidity of manufacturing islands by spin-coating, it is possible to obtain surfaces decorated with nanovolcano-like shapes that increase in height as the humidity of manufacturing increases. Surfaces with air bubbles inside these nanovolcanoes have a high water-repelling ability. It was shown that the samples with geometries $\frac{\sigma}{\xi} > 0.24$ were the most capable of storing air.

The nanoparticles inside the volcanoes could be agglomerated with a droplet of a suspension with nanoparticles located on the surface of selected samples. The sample was placed in the vacuum chamber, and the air was aspirated away. In this way, the bubbles escaped from inside the cavities, and the nanoparticles were aspirated into the interior. The mean diameter of the pores with HAp was $344 \text{ nm} \pm 102 \text{ nm}$, and $\frac{\sigma}{\xi}$ was $0.29 \pm 0.08$.

Whether hydroxyapatite was trapped inside nanovolcanoes or not, the surfaces could promote or inhibit cell adhesion and proliferation.

Further improvements and investigations can be enhanced with premicropatterning with lithographic methods to gain more control over the size and distribution of nanovolcanoes, and rinsing could benefit from the passivation of the surface to decrease the adhesion of excess nanoparticles. The utilization of nanoparticles with different functional properties would be another interesting follow-up.

4. Materials and Methods

4.1 Preparation of polymer blend solutions for spin-coating

Linear and amorphous GPC-grade polymer standards of poly(methyl methacrylate) (PMMA, 20 kDa, PDI 1.07) and polystyrene (PS, 20 kDa, PDI 1.07, 90 kDa, PDI 1.04, 200 kDa, PDI 1.04) were used as supplied by Sigma-Aldrich/Merck KGaA. The molecular weights of the polymer solvents, methyl ethyl ketone (MEK) and cyclohexane (C6H12), were supplied by
Sigma-Aldrich/Merck KGaA. Stock solutions of each polymer (100 mg/ml) were prepared in MEK. The solutions were stirred for 1 h at 37 °C before storing overnight in a refrigerator. Stock solutions and pure MEK were blended accordingly to obtain the desired composition, sonicated for 15 min, and stirred at 37 °C for 1 h before spin-coating. The blends, their molecular weight, and polydispersity are listed in Table 4. Blends with a mixture of two kinds of PS are referred to as bimodal, and those with one kind of PS are referred to as uniform.

<table>
<thead>
<tr>
<th>Mw property</th>
<th>2090200; 2575</th>
<th>2090200; 5050</th>
<th>2090200; 7525</th>
<th>2020200; 2575</th>
<th>2020200; 5050</th>
<th>2020200; 7525</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn [kDa]a)</td>
<td>173</td>
<td>146</td>
<td>118</td>
<td>155</td>
<td>110</td>
<td>65</td>
</tr>
<tr>
<td>Mw [kDa]a)</td>
<td>186</td>
<td>166</td>
<td>137</td>
<td>194</td>
<td>184</td>
<td>158</td>
</tr>
<tr>
<td>PDI b)</td>
<td>1.08</td>
<td>1.14</td>
<td>1.16</td>
<td>1.25</td>
<td>1.67</td>
<td>2.43</td>
</tr>
</tbody>
</table>

\[ M_w = (f_1 M_{w1}^2 + f_2 M_{w2}^2)/(f_1 M_{w1} + f_2 M_{w2}), M_n = f_1 M_{n1} + f_2 M_{n2}, f - \text{fraction of one of the polymers in \%}, \text{PDI = } M_w/M_n - \text{polydispersity index.} \]

4.2 Spin-coating procedure

Home-made spin-coating was used to precisely control the humidity in the chamber during the process. The spin-coating device is described in detail in ref.[38] SiO wafers, 300 μm thick, one side polished, Technolutions Sp. z o. o., were cut into 1 cm x 1 cm pieces, placed in an acetone bath for 1 h at 60 °C, and then dried in a stream of dry air. A series of concentrations from 15 mg/ml to 35 mg/ml were used. The humidity was monitored in real time by a sensor inside the chamber. The chamber was flushed with a 10 l/min stream of air with the desired humidity. A droplet of 20 μl of the solution was pipetted on a wafer and spun at 2700 rpm for 10 s.

4.3 Preparation of samples with NVs

The samples were dissolved in cyclohexane at 34 °C. A sample was immersed three times, mixed in a vessel with solvent, and dried in a stream of dry air. The samples were stored overnight. The samples were sterilized for 60 min with a UV lamp for cell culture.

4.5 Agglomeration of the HAp nanoparticles inside the nanovolcanoes

Hydroxyapatite, HAp nanoparticles were delivered by the Institute of High-Pressure Physics, PAS, which are distributed by the institute under the trade name GoHAP™, type 3.[56] The specific surface area, SSA, of the nanoparticles was 154 m²/g, and the density was 2.9334 g/cm³.
A 10% w/v aqueous suspension of HAp was prepared by adding nanopowder to a vessel with pure deionized water and stirring for a day. The suspension was then left for 6 h to suspend falling. The settled HAp suspension consistency formed a gel-like fraction on the bottom of the bottle. The thickened part of the suspension was pipetted onto the samples with NVs placed inside the exicator. Immediately after, the exicator was sealed, and the laboratory vacuum pump was turned on. The samples were left in a vacuum to dry. The remaining HAp was blown with a stream of compressed air, and then the samples were washed in an aqueous bath with constant stirring for 30 minutes. Autoclaved water and vessels were used for cell culture studies. HAp nanopowder was sterilized with a UV lamp for 1 h.

4.6 Characterization of the HAp suspension
The solid content of the HAP slurries was estimated using a moisture analyzer, Santorius MA 160-1 (Santorius, Germany). The average grain/agglomerate size in suspensions was estimated by a Zetasizer Nano-ZS (Malvern Instruments, Ltd., England) using the dynamic light scattering (DLS) technique according to the ISO 22412:2008 standard.

4.7 Imaging
Atomic force microscopy (AFM, Oxford Instruments/Asylum Research MFP3D Bio) was used for imaging. For tapping mode imaging, an Olympus OMCL-AC200TS scanning probe was used. For lateral force imaging (LFM), AppNano Hydra probes were used with a spring constant of $k = 0.284$. The scanning speed was 0.5 Hz, and the set point was 0.5 V. For bubble air-water interphase imaging, the Olympus TR400-PSA probe was used with nominal spring constant $k = 0.08$ N/m, the scanning speed was 0.3 Hz, and the set point was 0.3 V, the gain was set to 2. For scanning electron microscopy, a Zeiss Ultra+ SEM was used.

4.8 Contact angle measurement
The wetting contact angle was measured using a Data Physics OCA 20 goniometer using the sessile drop method.

4.9 Fourier Transform Infrared Spectroscopy
Fourier transform infrared spectroscopy, Nicolet 8700 FTIR, was used to investigate the chemical composition of the samples. The ATR attachment was used to collect the spectra over a range of 4000–400 cm$^{-1}$.  
4.10 Cell culture studies

The normal fibroblast line L929 (CCL-1) and osteosarcoma cell line MG-63 (CRL-1427) were used in the experiments. All cell lines were adherent cells. All cell lines were obtained from the American Type Culture Collection (ATCC). The MG-63 cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Thermo Scientific, Waltham, MA, USA). L929 cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI, Gibco). Culture media were supplemented with 10% (v/v) bovine serum albumin (Gibco) and 1% (v/v) penicillin-streptomycin (Gibco). Cells were cultured under standard conditions, i.e., 37°C, 5% CO₂, and 95% humidity. Before the cell culture experiment, the tested samples were sterilized with UV light for 30 minutes. Samples with a side length of 1 cm placed in multiwell plates were flooded with cell medium, and then a droplet suspended from 50k cells was seeded. Low cell density and high dispersion of seeding were obtained. The cells were grown for 72 h. Then, the cells were stained with a Live/Dead assay (Thermo Fisher Scientific) and investigated by confocal microscopy. For SEM imaging, samples were fixed with paraformaldehyde and dried with increasing ethanol concentrations.

Succinate dehydrogenase activity assay

L929 and MG-63 cells were seeded at a concentration of $5 \times 10^4$ cells/per NVs coated with HAp and well of 12-well plates (as a control). Cell counts were quantified using an EVE auto-reader (NanoEnTek, Waltham, MA, USA). Culture medium in a volume of 2 mL was added to all wells. In the study groups, NVs with HAp were also located in the well of the plate. After 72 h of incubation of the cells on polystyrene wells with NVs or without NVs, the mitochondrial activity of the cells was determined using the tetrazolium dye XTT (Roche Protocol, Mannheim, Germany). For this purpose, 150 µl of XTT labeling mixture was added to each culture well containing 300 µl of culture medium. The XTT labeling mixture was prepared by mixing the labeling reagent with the electron coupling reagent in a ratio of 1:50. The culture fluid was transferred to a new plate before reading. Absorbance results were read after 8 hours of incubation using a Tecan Infinite 200 microplate reader (Tecan, Durham, NC, USA) at 492 nm and a reference wavelength of 690 nm.

The activity was calculated as in eq. 5.

\[
\frac{A_{\text{sample}} - A_{\text{background}}}{A_{\text{control}} - A_{\text{background}}} \quad (5).
\]

Here, $A_{\text{sample}}$ is the absorbance of the test sample, $A_{\text{control}}$ is the absorbance of the control sample where cells were seeded only at the bottom of the well of the multiwell plate, and $A_{\text{background}}$ is the absorbance of the medium with the labeling mixture added.
4.11 Statistical analysis of surface geometry from image analysis

The cube-volume aspect ratio, CVAR, was used \( CVAR = \frac{2\pi r}{h} \) to determine how flattened the polystyrene islands were. Values close to unity mean that the base and height are similar. The flatter the islands are, the higher the CVAR value. The average radius \( r_i \) and height \( h_i \) of islands were obtained from AFM images by a mask threshold method. Statistical analysis of the AFM images based on the autocorrelation length was employed to find the characteristic length of the structures, referred to as the autocorrelation length, ACL, or \( \xi \), and the root mean square roughness \( \sigma \). By finding the ratio between the statistical height variation and the statistical length, \( \sigma/\xi \), it is possible to compare the steepness of various rough surfaces.\(^{47-49}\) This kind of analysis was even utilized to compare the steepness of the nanostructures to the steepness of real mountains.\(^{50}\) The parameters were obtained by approximating the autocorrelation function, ACF, of AFM images with a Gaussian-type distribution, eq. 6.\(^{57}\) The algorithms implemented in Gwyddion 2.5 software were used for analysis.

\[ C = \sigma^2 e^{-r^2/\xi^2} \]  

(6)

Supporting Information

Acknowledgments

The authors would like to kindly thank MSc Urszula Szałaj from the Institute of High Pressure Physics, PAS, for delivering HAp nanoparticles, which are distributed by the institute under the trade name GoHAP™. The authors would like to thank Dr. Adrian Chlanda for his help with film thickness measurements. This work was supported by the National Centre for Research and Developments [STRATEGMED3/306888/3/NCBR/2017] and Ministry of Science and Higher Education (the subsidy funds). The equipment used in the following research was funded by the Centre for Preclinical Research and Technology -CePT II from the Operational Program of the Masovian Voivodship (RPMA.01.01.00-14-8476/17-01).

Conflict of Interest

The authors declare no conflict of interest.
Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

References

The table of contents

Design of polymeric thin films with nanovolcanoes for trapping hydroxyapatite nanoparticles to promote or inhibit cell proliferation

Maciej Łojkowski*, Ewa Walejewska, Malwina Sosnowska, Agnieszka Opalińska, Kamil Grubczak, Sławomir Jaworski, Marcin Moniuszko, Wojciech Swieszkowski*

A strategy has been proposed for tuning the geometry of polymeric nanovolcanoes to store air bubbles while submerged underwater. Hydroxyapatite nanoparticles agglomerate into the cavities due to subpressure caused by vacuum. We have shown that cells will proliferate in the presence of nanovolcanoes with hydroxyapatite, and nanovolcanoes without hydroxyapatite will block proliferation.