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Fibroblast/fibrocyte: surface interaction dictates tissue reactions to micropillar implants

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Abstract

Micropillar technology has shown great promise for medical implants or sensors in recent years. To study the influence of surface topography on cellular responses, polydimethylsiloxane micropillar arrays with pillar spacing (20–70 μm) and height (14–25 μm) were tested on their ability to alter cell behavior both in vitro and in vivo. Interestingly, in vitro, we observe a distinct response for 3T3 fibroblasts and RAW 264.7 macrophages to the topographical cues tested. Attachment and proliferation of fibroblasts was substantially enhanced by increasing pillar height, while macrophage adherence is significantly diminished by reduced pillar spacing. When implanted in the subcutaneous cavity of BALB/c mice for 14 days, we find a prevailing trend with total cell recruitment number and capsule thickness increasing, as both pillar height and spacing rise. Collagen deposition and neoangiogenesis, two pivotal factors in granulation tissue maturation, are also observed to have a stronger response to the increase in both pillar height and spacing. In contradiction to our original hypothesis we observed that fibroblasts rather than macrophages are a key contributor to the in vivo outcome of micropillar arrays. Investigation into fibroblast activation however revealed that recruited fibrocytes, rather than resident fibroblasts, correspond with the in vivo outcome. The results from this work support the critical and, often, overlooked role of fibrocytes in tissue responses to biomaterial implants with varying topography.

Introduction

Micropillar arrays have been studied extensively in recent years as a potential tool for fabrication of sensors, actuators, and tunable wetting surfaces [1–2] for a variety of applications including proteomics, genomics, and metabolomics. To develop micropillar biosensors or implants, increasing research efforts have been placed on investigating the influence of micropillar topography on cellular responses such as migration [3–6], differentiation [7–9], and cell deformation [9–10] in vitro. These investigations have uncovered many interesting effects of micropillar topography on cellular reactions. However, partially due to the fact that these studies are carried out with different cell types and culture conditions, the micropillar-associated cellular responses are often inconsistent between studies. For example, cellular proliferation is not observed with some spatial pillar arrangements but is observed with others [3, 6]. Cell morphology and incorporation of pillars by cells have been shown to be influenced by both height and spacing. Furthermore recent evidence has linked both preferential differentiation [7–9] and malignant transformation [10] to cues governed by micropillar geometry. Finally and most importantly,

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the influence of micropillar topography on in vivo immune and tissue responses is mostly undetermined.

It is well established that macrophages (MΦs) play an important role of regulating foreign body reactions-associated cellular responses [11–13]. Many studies have shown that biomaterial implants prompt the activation of MΦs to release a variety of pro-inflammatory cytokines, such as Tumor Necrosis Factor- α , Interleukin-1 β , Platelet-Derived Growth Factor, Macrophage Inflammatory Protein 1- α , Monocyte Chemoattractant 1- α and Transforming Growth Factor- β [11–14]. The release of these cytokines is thought to be responsible to the fibrotic tissue formation, collagen formation and angiogenesis [13–16]. Recent study using micro- and nano-groove surfaces found that MΦs are responsive to surface topography above 500 nm [17]. However, it is mostly unclear whether topography in micrometer ranges would trigger different extent of MΦs responses both in vitro and in vivo.

Fibroblast proliferation and collagen production are the trademarks of fibrotic tissue reactions to biomaterials implants [18]. Activated fibroblasts, characterized by expression of α -SMA, are responsible to the increased collagen production, extracellular matrix secretion and implant encapsulation [19]. It is generally believed that tissue fibroblast expansion following injury or fibrotic disease results in a more active or contractile phenotype, myofibroblast. Although work has been done to identify the source of myofibroblasts participating in foreign body reactions the relative contributions from mechanisms such as epithelial-mesenchymal transition, tissue-derived, and bone-marrow derived mesenchymal stem cells remain up to some debate [19–22]. Recent studies have suggested that fibrocytes, a blood-born and migrating fibroblasts, play an important role of several inflammatory and fibrotic diseases, including pulmonary fibrosis, scleroderma, renal and hepatic fibrosis, and asthma [23–26]. The results from these studies and others reveal that fibrocytes may be recruited to the injured tissue by inflammatory stimuli [27–28]. The recruited fibrocytes are hyper-proliferative and produce excessive amount of collagen in tissue which lead to formation of scar tissue during wound healing responses [22, 27–28]. However, to the best of our knowledge, the potential role of fibrocytes in foreign body reactions has not been determined yet. The influence of micropillar topography on both in vitro and in vivo fibrocyte/ fibroblast behaviors also has not been characterized.

To investigate the effect of surface micropillar topography on foreign body reactions, a series of micropillar substrates made of polydimethylsiloxane (PDMS) with varying pillar height and spacing were fabricated and then tested in this work. Since MΦs and fibroblasts are main cells responsible for foreign body reactions, we first studied how micropillar substrates affect the adhesion and proliferation of MΦ RAW cells and 3T3 fibroblasts in vitro. Subsequently, double-sided micropillar substrates were implanted in the mice subcutaneous cavity for two weeks. The extent of in vivo cellular and tissue responses to various substrates was then evaluated using histological and immunohistochemical techniques. The results were used to assess the interplay of macrophages and fibroblasts and, most importantly, the mediating influence of micropillar topography on the balance of these cell responses for subsequent granulation and fibrotic tissue formation.

Materials and Methods

Materials

Polydimethylsiloxane (PDMS) was purchased from Dow Corning Corporation (Midland, MI). Dulbecco's modified Eagle medium (DMEM) was purchased from Sigma (St. Louis, MO). Fetal calf serum and antibiotics (100 U/mL penicillin, 100 ug/ml streptomycin) were obtained from Atlanta Biologicals (Lawrenceville, GA). Mouse 3T3-Swiss albino fibroblasts and RAW246.7 macrophages were obtained from ATCC (Manassas, VA). A live cell tracer

dye, carboxylfluorescein diacetate succinimidyl ester (CFDA-SE) was purchased from Invitrogen (Carlsbad, CA). Histological reagents including Haematoxylin, Eosin Y, and Masson's trichrome, were acquired from Sigma Aldrich Inc. (St Louis, MO). Antibodies against 11b (CD11b), alpha smooth muscle actin (α -SMA) and cluster of differentiation 45 (CD45) were procured from Santa Cruz Biotech (Santa Cruz, CA). Secondary antibodies coupled with FITC or Texas Red were bought from ProSci (Poway, CA). Nuclei stain - 4',6'-diamino-2-phenylindole dihydrochloride (DAPI) was obtained from Invitrogen (Carlsbad, CA).

Micropillar fabrication/ characterization

The micropillar arrays were fabricated from PDMS due to its ease of processing, and biocompatibility [29]. The surface topography was fabricated using a molding method in which the polymer was cast on a SU-8 mold with microholes [30]. The dimensions of pillars were set at $10 \times 10 \mu\text{m}^2$ with heights at either 14 or 25 μm (abbreviated as H_{14} or H_{25}) in a centered hexagonal array. Two arrangements of pillar orientation with varying inter spaces are studied at 20 and 70 μm (abbreviated as S_{20} or S_{70}). An outline for geometrical configurations of the pillar substrates is presented in Figure 1.

Single-sided micropillar arrays were fabricated in square sheets of $10 \times 10 \text{ mm}^2$. For in vitro cell culture only single sided pillar arrays were used. For in vivo studies, two single-sided pillar arrays of similar micropillar orientation were fashioned together back to back by applying a thin layer of liquid PDMS followed by curing process. Characterization of the micropillar substrates is performed by SEM imaging analysis with a Hitachi 3000N Scanning Electron Microscope (Hitachi High Tech Inc, Tokyo, Japan). For both in vitro and in vivo testing, a $5 \times 10 \text{ mm}^2$ array was sectioned and used for each type of micropillar substrates along with the control PDMS films of smooth surfaces. Micropillar substrates were sterilized in 70% ethanol followed by 20 minutes UV exposure in a culture hood prior to in vitro and in vivo testing.

In vitro cell proliferation on micropillar substrates

3T3 fibroblast cells and RAW246.7 cells were cultured to 75% confluence maintained at 37°C in a humidified atmosphere of 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum and 1% antibiotics. For each type of cell and pillar array, a low density (2000 cells/10ul) of cells was seeded on $5 \times 10 \text{ mm}^2$ samples so that the growth and morphology of the cell could be studied. After incubation for 4 hours, supernatants were removed and the adherent cells were then stained with a cell tracer dye CFDA-SE as previously described [31]. The numbers of adherent cells were then quantified under fluorescence microscope (Leica, Wetzlar, Germany) on 8 different areas covering the whole pillar substrate surfaces. On each subsequent day, cell growth was monitored by re-staining with tracer dye and quantifying the number of adherent cells.

In vivo implantation of micropillar substrates

To test the influence of surface topography on tissue reactions, micropillar substrates were implanted in the dorsal subcutaneous region of BALB/c mice (~25gm body weight). After Implantation for 14 days the animals were sacrificed. The implants and surrounding tissue were isolated and kept in freezing medium Tissue-Tek OCT (Sakura FineTek, Torrance, CA, USA) at -80°C for histological evaluation. Animals were cared for in compliance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Arlington.

Histological and immunochemical analyses

All tissue samples were sectioned at 10 μm on a cryostat (Leica CM1850, Leica Microsystems, Wetzlar, Germany) and collected on poly(l-lysine) treated slides. To assess the overall gross cellular response to the implants, some of the tissue sections were stained with haematoxylin and eosin Y (H&E) or Masson's trichrome. Overall cellular response was assessed by total cell count and thickness of surrounding fibrotic capsule measured using NIH ImageJ on captured microscopic images as established earlier [32–33]. Collagen deposition was observed and similarly assessed from Masson's trichrome stains. The relative level of collagen staining was assessed using ImageJ processing. To assess different types of cellular responses, some tissue sections were stained with inflammatory cell marker CD11b, fibroblast marker α -SMA, or fibrocyte markers CD45/ α -SMA. For clear assessment of the cells, a DAPI stain (which stains the cell nuclei blue) was also applied during IHC. All histological imaging analysis was performed on a Leica microscope (Leica, Wetzlar, Germany) and processed using NIH ImageJ [32–33].

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. The statistical significance between groups or sets of data was calculated using a One Way ANOVA analysis with a Bonferroni comparison. Data are taken to be significant when P values of $p < 0.05$ were obtained. Linear regression analyses was used to determine the relationship between in vitro and in vivo results, as well as assess the correlation between cell responses in vitro and over all in vivo results. The coefficient of determination (R^2) was calculated providing a measure of correlation.

Results

Micropillar characterization

A general overview of the fabricated PDMS micropillar substrate is shown in figure 1. We confirmed that the pillars maintained heights at either 14 or 25 μm (labeled as H_{14} or H_{25}) in a centered hexagonal array (figure 1A, B & C). Two arrangements of pillar orientation with varying inter spaces are studied at 20 and 70 μm (Labeled as S_{20} or S_{70}). An initial sample with dimensions of 20 by 18 μm , width by length on center, as shown in schematic 1D was designed with the typical mammalian cell in mind (approximately 10 μm in diameter). In an attempt to alter the cellular response, an additional pillar orientation was tested with wider spacing, allowing cells to attach and spread throughout the interspatial pillar regions. The two different dimensions of the substrates utilized in this study were determined based on SEM images (figure 1E). Samples were labeled based on their physical characteristics by spacing and height (S, H) for example ($S_{20}H_{25}$) refers to pillar interspacing of 20 μm and pillar height of 25 μm .

In vitro cellular morphology and proliferation

Interestingly, the presence of micropillars was found to reduce the adherence, spreading and proliferation of M Φ - RAW cells (figure 2A). Specifically, substrates with short and small-spaced micropillars ($S_{20}H_{14}$) were observed with less cells attached per area in comparison to PDMS control and other micropillar substrates. In addition, short micropillar substrates ($S_{20}H_{14}$, $S_{70}H_{14}$) are also found with lower M Φ attachment compared to tall micropillar substrates of the same spacing ($S_{20}H_{25}$, $S_{70}H_{25}$), respectively. Furthermore, SEM images show that M Φ sparsely reside on the tops or sides of pillars on the S_{20} arrays while they surround and proliferate throughout the interspatial regions of the S_{70} arrays (figure 2A). The quantitative cell adherence results support that there is a reverse trend with increasing micropillar height (14 $\rightarrow$ 25 μm), spacing (20 $\rightarrow$ 70 μm), or both dimensions (Figure 2B). It

should be noted that $S_{20}H_{14}$ is the only group of the substrates to show statistically different from the smooth surface control.

In contrast to the MΦ response, the 3T3 fibroblasts show a varying response based primarily on the pillar height (figure 3A). Specifically, as micropillar height increases, the cells attach and spread across the surface more easily. On small-spaced tall-pillar substrates - $S_{20}H_{25}$, most of fibroblasts spread across micropillars. However, on large-spaced tall-pillar substrate - $S_{70}H_{25}$, colonies of fibroblasts grow around and sometime overtake the micropillars. Less cell proliferation is observed on the control samples and substrates with lower micropillars ($S_{20}H_{14}$, $S_{70}H_{14}$) (figure 3A). Such observations are supported by our quantitative results where 3T3 fibroblast attachment and proliferation is significantly influenced by the presence of the higher micropillars in comparison to the control (figure 3B) ($P < 0.01$). The differences between various spacing however were not significant.

In vivo total cellular response

To determine whether micropillar physical characteristics would also affect the extent of MΦ and fibroblast recruitment and subsequent fibrotic tissue reactions, double-sided micropillar substrates were implanted in the subcutaneous cavities for two weeks. By analyzing the tissue response to micropillar implants, we find that the presence of micropillars augments total cell recruitment over control (figure 4A) similar to the fibroblast in vitro responses mentioned above. We observed that the higher pillar substrates ($S_{20}H_{25}$, $S_{70}H_{25}$) gathered more granulocytes and fibroblasts surrounding the pillars while the lower pillar substrates ($S_{20}H_{14}$, $S_{70}H_{14}$) accumulated small numbers and layers of inflammatory and fibrotic cells (figure 4A). By quantifying the cellular density within the capsule, we found that total cell density increases with pillar height (figure 4B). The control maintains the lowest response while the cellular densities of the higher pillar substrates ($S_{20}H_{25}$, $S_{70}H_{25}$) are increased $>50\%$ over the control. These results suggest a preferential accumulation of cells between host tissue and micropillar substrates. We also observed that pillar height and spacing exerted similar augmenting effects on capsule thickness (figure 4C). Compared with PDMS controls, substrates with large-spacing ($S_{70}H_{14}$, $S_{70}H_{25}$) exert significant increase of capsule thickness. However, the differences of capsule thickness between control, $S_{20}H_{14}$ and $S_{20}H_{25}$ were not statistically significant.

Relationship between in vitro cell adherence and in vivo cellular responses

It is generally believed that MΦs have greater impact on tissue reaction to biomaterial implants. To test this hypothesis and identify the critical cells responsible for in vivo outcomes, we first determine the correlation between cell: substrate interactions in vitro and fibrotic tissue responses in vivo. Very surprisingly, we find that the relationships between macrophage responses in vitro and fibrotic reactions in vivo are poor (linear correlation to total cell recruitment $R^2=0.02$ and to capsule thickness $R^2=0.31$) (figure 5A-B). On the other hand, there is a good relationship between fibroblast responses in vitro and fibrotic reactions in vivo (linear correlation to total cell recruitment $R^2=0.77$, and to capsule thickness $R^2=0.74$) (figure 5A-B). Contradicting to our hypothesis, these results suggest that the responses of fibroblasts, but not MΦs, dictate the overall outcome of tissue reactions to pillar substrate.

Effect of pillar height and spacing on implant interface integration

The formation of granulation tissue and angiogenesis are common measures of integration in the foreign body response. To identify the influence of pillars on cellular integration at the implant interface, we examine how pillar height and spacing affect tissue reactions, including granulation tissue formation and angiogenesis. We thus examine how pillar height and spacing affect the granulation tissue formation at the interface as well as the

neoangiogenesis within the capsule. Very interestingly, we find that the accumulation of granuloma cells at the interface increases slightly with higher pillars and larger spacing - $S_{70}H_{25}$ (figure 6A). Additionally, the same substrates were found to significantly enhance vascular neoangiogenesis (figure 6B). The relative degree of collagen production is similarly observed to augment with increasing pillar height from 14 to 25 μm with both pillar spacing dimensions while $S_{70}H_{25}$ pillar array was found to have significant more collagen production than the control (figure 6C).

Effect of pillar height and spacing on inflammatory and fibrotic cell accumulation

Although our results show that micropillar topography affect tissue responses, the cellular mechanisms governing such topography-mediated cellular responses are not clear. To identify the cell type responsible to micropillar-associated tissue responses, we focused on CD11b+ inflammatory cells and α -SMA+ fibroblasts since both types of cells were found to be the major cell types present in the fibrotic capsule surrounding biomaterial implants. Staining for CD11b+ inflammatory cells shows a significant increase over the control for the micropillar arrays. There is however no significant difference observed for pillar height or pillar spacing alone (figure 7A). On the other hand, fibroblasts stained with α -SMA (representing all types of the fibroblasts) show increased recruitment with spacing and height, though such difference is only significant with the $S_{70}H_{25}$ substrate (figure 7B). These results suggest that micropillar topography-mediated cellular responses are fibroblast-dependent. Since the correlation between α -SMA+ fibroblast cell numbers and total cell numbers are not significant (data not shown), it is likely that sub-type of fibroblasts is responsible to topography-associated tissue reactions.

Fibrocytes are unique circulating fibroblasts which participate in pathogenesis of many inflammatory and fibrotic diseases. To investigate whether fibrocytes may play a role in topography-dependent cellular responses, we first observed the presence of fibrocytes (α -SMA+/CD45+) in the interface of micropillar substrates. Indeed, we find that the pro-fibrotic substrates - $S_{70}H_{25}$ - accumulate more fibrocytes than control surfaces (figure 7C, YOU NEED TO LABEL YOUR PHOTOS). Quantitative results show that tall micropillar substrates ($S_{20}H_{25}$ and $S_{70}H_{25}$) prompt the most fibrocyte recruitment over controls and short micropillar substrates (figure 7D). Furthermore, a correlation between the in vivo total cell number and the recruited fibrocyte cell number shows a near linear trend ($R^2=0.822$) indicating a strong potential role of fibrocytes in micropillar topography-mediated tissue responses (figure 7E).

Discussions

To assess the tissue responses to micropillar arrays, substrates with different pillar heights and spacing were incubated with two main cell types responsible for foreign body reactions, MΦs and fibroblasts [13, 15, 18]. Surprisingly, we have uncovered that different types of cells prompt varying responses to the same micropillar substrates. Specifically, we find that the increase of pillar height, but not pillar spacing, substantially enhance fibroblast proliferation while MΦ proliferation is inhibited by substrates with small pillar spacing, but not pillar height. Pillar height-dependent fibroblast proliferation is in agreement with several recent observations. First, several studies have revealed that fibroblasts alter their mode of attachment, cell shape, and migration patterns in response to topographical cues [3–6]. Second, previous study showed that micropillar height from 2 μm to 10 μm provided greater focal adhesions and increased the growth rate of fibroblasts [6]. Using microchannel patterned substrates, MΦs were found to preferentially accumulate on rough surfaces, and also to respond to increased width of μm range microchannel gratings [12, 17]. However, the inhibitory growth of MΦs by small spaced micropillars has not been documented before. Furthermore, MΦs were found to be insensitive to grooves of less than 500 nm, preferring to

respond to grooves of 500 nm-2 μ m [17]. It is not clear why fibroblasts are more reactive to micropillar height while M Φ s growth is slowed down by small-spaced pillars. It is possible that tall micropillars provide more spatial surface area for extracellular matrix production and subsequent fibroblast activation as indicated in the early works [3–8, 34–35]. However, it is not clear how surface micropillar topography influence immune reactions or combined M Φ /fibroblast responses in vivo.

Using a subcutaneous implantation model in mice, we find that all micropillar substrates significantly increase the recruitment of inflammatory (CD11b+) cells and fibrotic (α -SMA +) cells toward implant surfaces. However, the highest extent of cell recruitment and capsule tissue formation was found associated with micropillar substrate with high pillar height and spacing. Since all micropillar substrates prompt similar extent of inflammatory cell recruitment, it is likely that certain surface topography (70 μ m spacing and 25 μ m pillar height) substantially increases cell activation and subsequent tissue reactions, including collagen production and angiogenesis. The varying tissue responses surrounding micropillar responses are likely caused by surface topography, due to increased cell adhesion and preferential accumulation at the interface. It has also been shown that substrates bearing different surface chemistry but similar topography prompt similar inflammatory responses in rats [17] indicating topography as a major contributor. Furthermore, to compare the in vitro and in vivo results, we find a poor relationship to the macrophage growth rate, which contradicts the general hypothesis that macrophages are critical to fibrotic tissue reaction to biomaterials implants [12]. In contrary and very interestingly, the in vitro fibroblast growth has a good linear relationship ($R^2 > 0.7$) with the in vivo cellular and tissue responses. These promising and surprising findings reveal un-recognized importance of fibroblast in tissue responses to micropillar implants. Indeed, many recent observations indicate that fibroblast-like cells play a critical role in wound healing [27, 36]. Furthermore, the fibroblast activation has been linked to the increased granulation tissue and neoangiogenesis [37–39]. It is not clear how surface topography affects fibroblast activation.

To uncover the mechanism(s) of surface topography-mediated fibroblast activation, we first tried to identify the sources of implant-associated “fibroblasts”. Based on universal fibroblast marker- α -SMA, we find that micropillar surfaces prompt similar extent of fibroblast recruitment. Since substantial recent studies have found that “fibrocytes” played in an important role of promoting inflammatory and fibrotic reactions in pulmonary fibrosis, scleroderma, renal and hepatic fibrosis, and asthma [23–26], subsequent analyses were carried out to investigate the influence of surface topography on fibrocyte responses. Indeed, immuno-staining for coexpression of CD45 and α -SMA to identify fibrocytes revealed a synergistic effect to both pillar height and pillar spacing. Equally important, our analyses also uncover a good linear relationship between fibrocyte numbers and total in vivo cell recruitment. While the complexity of the mechanisms involved remain undetermined we hypothesize that the recruited fibrocytes mediate the tissue response to changing to pillar dimensions. Fibrocytes have been implemented in the healing process releasing chemokines such as, Interleukin-6, Platelet-Derived Growth Factor, and Insulin Growth Factor-1, as well as influencing tissue repair through increased secretion of extracellular matrix components such as vimentin, fibronectin, collagen I and collagen III [22, 27, 40]. Furthermore the degree of recruitment frequency of bone marrow derived fibrocytes has been shown to be dependent on the tissue microenvironment [28]. It is likely that the microenvironment created by the pillars, especially with increased height and spacing, has a similar effect on the fibrocyte response, as in wound healing, which in turn produces a greater tissue response.

In this study we have identified unique knowledge on the characteristics of micropillar height and spacing on the effect of the cellular response. Specifically, tall pillars and large

pillar spacing provide a powerful microenvironment cue for the activation and proliferation of fibrocytes and fibroblasts. Furthermore, the extent of micropillar-associated fibroblast responses in vitro may provide reasonable prediction of tissue responses to the same substrates. The activated fibrocytes may be responsible for the excessive fibrotic tissue reactions and angiogenesis surrounding the implantation sites. We are also aware the current study does not exclude the participation of macrophages in micropillar-associated tissue responses. Further studies of interactions between micropillar topography and fibroblast/fibrocyte responses may lead to the generation of optimal micropillar arrays to achieve cell growth and angiogenesis that favor in vivo regeneration and tissue integration.

Conclusions

The effect of pillar height and spacing on the in vitro and in vivo response of fibroblast and macrophage cells was investigated. Using an in vitro model, we found that the increase of pillar height (but not pillar spacing) enhance fibroblast proliferation while the decrease of pillar spacing (but not pillar height) reduce macrophage accumulation. In spite of these extreme cellular responses in vitro, all pillar substrates prompt distinct tissue responses in vivo. By comparison with control, micropillar substrates with high pillar height and big pillar spacing prompt the strongest fibrotic tissue reactions reflected by collagen production, cell accumulation and angiogenesis. We find that fibrocytes, a subset of highly proinflammatory fibroblasts, play an important role in micropillar-associated tissue responses in vivo. In addition, there is a good relationship between fibroblast proliferation in vitro and fibrotic tissue reactions in vivo. We believe that the extent of in vivo tissue responses can be manipulated by controlled the parameters of pillar spacing to pillar height. More in-depth studies on the parameters of pillar dimension and density on cellular responses would help the future development of micropillar sensors or devices for in vitro or in vivo applications.

Acknowledgments

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Figure 1. SEM images of micro-pillars from lateral views demonstrating the morphology and alignment of pillars with a height of 25 μm and a diameter of 10 μm (A–B), and (C) top view of pillars illustrating consistency of design and pillar head diameter of 10 μm. Schematic drawing (D) of 20×18 μm pillar design. All designs have a similar orientation with differences in pillar spacing and pillar height as shown in table (E). Pillar height was tested at 25 and 14 μm. The scale bars for (A–C) correspond to 30 μm.

Figure 2.

SEM images of RAW246.7 cells cultured for 3 days in vitro on the PDMS control and pillar arrays (A). The S₂₀ micro-pillar substrates show clusters of cells attaching on or between pillars. The S₇₀ arrangements shows cells in the interspaces often accompanied with cells around or nearby pillars. Increased pillar height samples (S₂₀H₂₅ and S₇₀H₂₅) show increased attachment to the pillars and subsequent large colony formations. Arrows show pillars that are surrounded or covered by cells. Figure 3B presents a histogram of macrophage growth rate showing the mean $\pm$ one standard deviation at 3 days in culture. Significance was calculated with a One Way ANOVA -Bonferroni comparisons test and taken to be slightly significant *P<0.05, or very significant **P<0.01. The scale bar for SEM images in figure A corresponds to 25 μ m.

Figure 3.

SEM images of 3T3 cells cultured for 3 days in vitro on the PDMS control and pillar arrays (A). The S₂₀H₁₄ and S₂₀H₂₅ micro-pillar arrays show cells stretching across the tops of the pillars, with consistently larger cell clusters on the taller 25μm pillars. The S₇₀H₁₄ and S₇₀H₂₅ arrangements show cells near or around pillars. There are frequently more cells around the taller 25μm pillars with greater matrix secretion. Arrows show pillars that are surrounded or covered by cells. Figure 2B presents a histogram of 3T3 growth rate showing the mean $\pm$ one standard deviation at 3 days in culture. Significance was calculated with a One Way ANOVA -Bonferroni comparisons test and taken to be slightly significant *P<0.05, or very significant **P<0.01. The scale bar for SEM images in figure A corresponds to 50 μm.

Figure 4. H&E images show the cellular response to control and each pillar array as stated above (A). Graphs present the means $\pm$ standard deviation of average cell numbers (B) and fibrotic capsule thickness (C) for micro-pillar groups. Significance was calculated with a One Way ANOVA -Bonferroni comparisons test and taken to be slightly significant * $P < 0.05$, or very significant ** $P < 0.01$. The scale bar for H&E images corresponds to 50μm.

Figure 5.

Correlations of In Vitro cell proliferation and In Vivo cell recruitment. A+B show 3T3 Fibroblast linear correlations to In Vivo cell number ($R^2=0.77$) and Fibrotic capsule thickness ($R^2=0.74$) respectively. C+D show RAW Macrophage linear correlations to In Vivo cell number ($R^2=0.02$) and Fibrotic capsule thickness ($R^2=0.31$) respectively.

Figure 6.

Effect of pillar height and spacing on granulation tissue formation, angiogenesis, and collagen production. Graphs present the effect of substrates on the granulation tissue thickness (A), the number of blood vessels throughout the capsule (B), and the relative percent of collagen deposition (C).

Figure 7.

Effect of pillar height and spacing on cellular recruitment. Graphs present the effect of substrates on the inflammatory response (measured by number of CD11b⁺ cells [A]) and fibrotic response (measured by number of αSMA⁺ cells [B]). IHC images (C) show the positive identification of fibrocytes (yellow) by overlay of CD45⁺ (green) and αSMA⁺ (red) cells with an included DAPI nuclear stain (blue). A graph of the corresponding fibrocyte response is presented in figure (D). A correlation of in vivo total cell number and identified fibrocyte cells (E) shows a near linear trend with $R^2=0.822$.