Development of a refined ex vivo model of peritoneal adhesion formation, and a role for Connexin 43 in their development

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Research Article

Abstract

Despite many advances across the surgical sciences, post-surgical peritoneal adhesions still pose a considerable risk in modern-day procedures. We have developed a novel mouse peritoneal strip ex vivo adhesion model which may serve to bridge the gap between single cell culture systems and *in vivo* animal drug testing for the assessment of potential anti-adhesion agents, and study of causality of the process.

We investigated the optimal conditions for adhesion formation with mouse peritoneal tissue strips by modifying an existing ex vivo rat model of peritoneal adhesions. We assessed the impact of the following conditions on the formation of adhesions: contact pressure, abrasions, and the presence of clotted blood.

Macroscopic adhesions were detected in all mouse peritoneal strips exposed to specific conditions, namely abrasions and clotted blood, where peritoneal surfaces were kept in contact with pressure using cotton gauze in a tissue cassette. Adhesions were confirmed microscopically. Interestingly, Connexin 43, a gap junction protein, was found to be upregulated at sites of adhesions.

Key features of this model were the use of padding the abraded tissue with gauze and the use of a standardised volume of clotted blood. Using this model, peritoneal strips cultured with clotted blood between abraded surfaces were found to reproducibly develop adhesion bands at 72 hours.

Introduction

Post-surgical peritoneal adhesions pose a considerable risk in modern-day procedures. Peritoneal and pelvic adhesions are a major cause of infertility [1]. Adhesions are a major complication of abdominal surgery, resulting in acute and chronic pain in patients [2]. Despite many advances across the surgical sciences, post-surgical peritoneal adhesions still plague even the most experienced surgeons. Various experimental models can be employed/studied to assess the efficacy of anti-adhesion agents in preventing post-surgical peritoneal adhesions. However, the models are inherently variable and require large numbers of animals per treatment group to ensure the reliability of the results. In addition to this are the requirements for calibration of each model, testing of multiple treatments and the comparison of dose-responses, which all further increase the number of animals used and the related costs.

Cell culture systems may represent a cost-effective option for initial screening of potential anti-adhesion agents. Such systems have the capacity for high throughput testing of multiple conditions and can help shed light on how cells function and respond to stimuli. For instance, cultures of fibroblasts taken from either normal or 'adhesion-prone' peritoneum may be a useful method to determine the potential of agents in limiting, arresting, or rescuing the adhesion phenotype [3-5]. However, single cell systems have their limitations. They are isolated cultures which neglects the effects of the neighbouring cells and the extracellular matrix that are present *in vivo*. The macro environment may influence the response to various stimuli [6] or alter cell fate differentiation and function [7]. Furthermore, long-term effects of drugs are challenging to assess using cell culture-based systems, as confluence often limits the duration

of study. However, 3D cultures have lower proliferation rates and may be better suited [7]. There remains a demand for new models to bridge the gap between single cell culture systems and *in vivo* animal drug testing for the assessment of potential anti-adhesions agents, and study of causality of the process.

Ex vivo tissue culture systems

One potential solution is the use of explanted tissue cultures. Saed and colleagues described an *ex vivo* rat model of peritoneal adhesion formation [8]. In this model, an abrasion injury was first performed on strips of tissue comprising peritoneum and its overlying abdominal muscle that had been harvested from rats. Following this, clotted blood from the same animal was placed on the abraded portion before the strips were folded in half and the ends sutured to prevent unfolding. The folded strips were then cultured for up to 72 hours. Each strip usually produced a single adhesive band that could be detected as early as 24 hours. The adhesions generated under these conditions were found to be consistent with the clinical observations of "Type 1B adhesions". This subtype is classified as "*de novo* adhesions that are produced at sites where a surgical procedure was performed and previously clear of adhesions" [9]. The adhesive bands generated in the rat model were described as dense, opaque, and vascularised.[8] This appeared to be an encouraging model, although several variables are not standardised (e.g. the quantity of blood and the abrasion injury). Other techniques/approaches in the model may introduce variability. For example, the suturing of the folded tissue strips to keep the ends from unfolding could introduce additional grasping and/or ischaemic injury.

The size of the rat peritoneal cavity allows multiple strips to be obtained from each rat. This allows multiple arms of a study to be tested on strips obtained from the same animal. This presents an advantage over *in vivo* methods where it is not possible to test multiple liquids, gels, or barriers in a single animal [10]. Furthermore, paired dose-response comparisons and intra-animal paired comparisons for different test articles can be accomplished [8]. Another advantage of the model is that it can be monitored and sampled following identification of desired events, such as vascularisation or adhesion creation. This allows the development of adhesions to be characterised and the effect of test articles on these processes to be properly assessed. With all these considerations taken into account, the rat model represents a cost-effective option for assessing the efficacy of a wide range of test articles.

Materials And Methods

Ex vivo media preparation

To prepare 1 L of culture media, the components and supplements were combined as listed in **Supplementary Table 1**. This initial mixture was then adjusted to a pH of 7.1 to 7.3 before the final volume was brought up to 1 L with deionised water. The prepared media was then filter-sterilised, and aliquots were stored at 4°C.

Tissue collection

Immediately preceding euthanasia with CO_2, fur around the BLK6 mouse peritoneum was removed using an electric trimmer and depilatory cream. After euthanasia, blood was collected by cardiac puncture. To standardise the amount of clotted blood, 200 μL of freshly harvested blood was left on ice to clot. The peritoneum site was sterilised with three rounds of 7.5% (v/v) providone-iodine solution and 70% (v/v) alcohol. The abdomen was harvested and full thickness peritoneal tissue strips (1.0 x 2.0 cm) comprised of the skin, underlying muscle, and peritoneum, were collected from the same mouse (**Fig. 1**). Blood was collected and stored in phosphate buffered saline supplemented with 3% Fetal Bovine Serum (HyClone, USA).

Induction of ex vivo adhesion formation and experimental design

The approach to induce *ex vivo* adhesion formation was modified from a published method [8], using mice (instead of rats; summarised in **Fig. 1**). In brief, harvested mouse peritoneal tissue strips in culture medium were rinsed in 70% (v/v) alcohol for 3 seconds and transferred to washing buffer A and B (**Supplementary Table 1**) for 3 minutes each. Experimental conditions of abrasion trauma and/or clotted blood were explored and are summarised in **Table 1**. Under conditions involving abrasion trauma, the peritoneum on the tissue strips was abraded 20 times evenly end-to-end by passing scraping a sterile size 10 scalpel in one direction with the sharp side down. Care was taken not to introduce any additional trauma, and the edge of the tissue which had been grasped by forceps was trimmed away before culture. In experimental conditions with clotted blood, 200 μL of clotted blood was added to the abraded site. Peritoneal strips without abrasion and/or clotted blood were used as controls.

Strips were folded in half with peritoneal surfaces facing each other and padded with sterile cotton gauze before being placed in a sterile tissue histology cassette. The gauze provided pressure, which ensured abraded surfaces remained in contact with one another without the need for sutures. Cassettes were placed in a 100-mm cell culture dish, fully submerged with culture media and incubated at 37°C, 5% CO_2 for up to 72 hours. At 72 hours, strips were gently unfolded and inspected thoroughly for the presence of adhesion bands (**Fig. 2**).

Table 1 Exploring conditions for inducing *ex vivo* adhesions

Conditions	Abrasion	Blood
1	+	+
2	+	-
3	-	+
4	-	-

n=5 each, 2 experimental repeats

Tissue processing sectioning and staining

Tissue samples were collected and stored in 4% paraformaldehyde at 4°C overnight. Tissues were transferred into 70% (v/v) ethanol and stored at 4°C overnight. They were processed using HistoCore PEARL (Leica, Germany) tissue processor and embedded in paraffin with the HistoCore Arcadia C (Leica, Germany) then sectioned at 5 μm using a Leica RM2245 microtome (Leica, Germany), mounted on Polysine® slides and dried at 40°C for at least an hour before staining. Slides were loaded into Leica Autostainer XL (Leica, Germany) and at the end of the H&E programme, glass coverslips were mounted onto the slides using Organo mounting medium (Sigma, USA) and dried at room temperature (RT). Alternatively, slides were cleared with two changes of Clearene, before hydrating successively in 100%, 95%, and 70% (v/v) ethanol gradients and deionised water. Slides were immersed in Picro-Sirius red (Abcam, United Kingdom) for 1 hour, then transferred into two changes of acetic acid followed by two changes of 100% (v/v) ethanol, cleared with two changes of Clearene (Leica, Germany) before glass coverslips were mounted onto the slides using Organo mounting medium (Sigma, USA) and dried at RT.

Immunofluorescence staining

Slides were cleared with two changes of Clearene, before hydrating successively in a series of ethanol gradients and PBS. Depending on the antibody, either heat-induced or proteolytic-induced antigen retrieval methods was performed. If no antigen retrieval was required, slides were immersed in permeabilisation/blocking buffer (0.3% (v/v) Triton-X100, 1M Lysine in PBS) for 1 hour at RT before incubation in antibody buffer (1% (w/v) BSA in PBS) for 45 minutes. Sections were immunolabeled with primary antibody overnight at 4°C. Connexin 43 (Cx43) (C6219 Sigma) 1:1000, Alpha smooth muscle acting (αSMA) (AB5694 Abcam) 1:500. Negative controls without primary antibody were included. Tissues were washed twice, 3 minutes each, in washing buffer (0.05% (v/v) Tween-20 in PBS) before 1 hour incubation at RT with secondary antibody Goat anti-rabbit AF488 or AF555 1:500 (A11008, A21422, ThermoFisher) then washed twice, 3 minutes each, before staining with DAPI (Life Technologies, USA) 1:10000, washed twice, 3 minutes each, in PBS prior to applying Citiflour[TM] AF1 (Electron Microscopy Sciences, London) mounting medium before cover-slipping.

Brightfield and confocal microscopy

Stained haematoxolin and eosin and picrosirus red (H&E and PSR) tissues were automatically scanned at 20X with an AxioScan.Z1 slide scanner (Zeiss, Germany). Scaling per pixel is 0.22 μm by 0.22 μm. For the immunohistochemical stained tissue, Z-stacks of 10 optical sections were acquired on a confocal microscope TCS SP8 (Leica, Germany) with a 40X 1.2NA Plan-Apochromat or a 63X 1.32NA oil objective. Fluorophores were excited sequentially using a 405 nm, 488 nm and 532 nm wavelength. The images were acquired at 8-bit, 1024 x 1024 pixels. All images were captured with identical laser and detector settings to allow for direct comparison.

Results

Development of *ex vivo* adhesions required the presence of contact, clotted blood and abrasion.

Peritoneal strips with clotted blood were cultured in the presence and absence of a cotton gauze (n=7 per group). Adhesions were detected in all the peritoneal strips cultured in the presence of the gauze. In contrast, only 57% (4 out of 7) of the strips that were cultured without gauze resulted in adhesions. Subsequent cultures were performed in the presence of gauze.

Four conditions (with or without abrasion and with or without blood, n=5 per condition; **Table 1**) were explored to determine which impacted the development of adhesions in the murine peritoneal strips. No adhesions were observed in conditions with no abrasions, regardless of the presence or absence of clotted blood. No adhesions were observed in conditions with abrasions but no clotted blood. However, adhesions were observed in strips with abrasions and cultured with clotted blood (5/5; **Fig. 2**). At least one adhesion band was detected macroscopically, although the total number of adhesion bands varied between peritoneal strips. The detected bands showed variation in strength and position along the abraded portion **(Fig. 2)**. Overall, we found that the combination of contact between peritoneal surface, abrasion trauma, and clotted blood was essential for adhesion development in this mouse *ex vivo* adhesion model.

Comparison of peritoneal strips cultured under different conditions

Histological differences between peritoneal strips cultured under the different conditions (n=5, per condition, Table 1) were further investigated (Fig. 3). In peritoneal strips cultured with clotted blood between the abraded surfaces (condition 1), areas of adhesions were identified in certain regions of juxtaposed mesothelium as described (Fig. 3ai and ii). In addition, the mesothelium appeared to be continuous along the strip, suggesting a certain degree of healing from abrasion trauma (Fig. 3a). However, the thickness of the mesothelium varied along the strip (Fig. 3aiii). In contrast, when peritoneal strips were abraded without the placement of clotted blood (condition 2; Fig. 3b), we observed discontinuous mesothelium with poor tissue integrity. While other regions of mesothelium along the same peritoneal strip appeared thick. However, when clotted blood was placed in the strip without abrasion (condition 3; Fig. 3c), the mesothelium was continuous but remained mostly thin. When both the placement of clotted blood and abrasion were absent (condition 4; Fig. 3d), a thin continuous mesothelium was observed across the entire strip in the absence of abrasion and clotted blood. These observations suggest that peritoneal strips cultured with clotted blood between the abraded surfaces (condition 1) were the most ideal conditions for generating ex vivo adhesions.

Microscopic assessment of adhesion formation/bands

Eosinophilic matrices were observed in the adhesions formed between certain regions of juxtaposing mesothelium along the strip **(Fig. 4ci and ii)**. Further histological assessment revealed these matrices had collagen deposition **(Fig. 4di and ii)**. In addition, we found the surrounding mesothelia peripheral to the matrices expressed high levels of αSMA, suggesting mesothelial-to-mesenchymal transition and sub-mesothelial fibroblast activation (Sandoval et al., 2016) **(Fig. 4ei and ii)**. We also detected elevated levels of Cx43 protein in mesothelial cells and sub-mesothelial cells in the regions of the peritoneal strip where adhesions were observed **(Fig. 4fi and ii)**.

Development of *ex vivo* adhesions over time

Cultures of peritoneal strips with placement of clotted blood between abraded surfaces were harvested at progressive time points (n=5, per time point) and examined for the development of *ex vivo* adhesions. In general, the discontinuous mesothelium caused by the abrasion trauma healed over time, becoming continuous by 72 hours.

The mesothelium at 0 and 6 hours were not significantly different, being thin and discontinuous along the entire strip (**Fig. 5a and b**). At 0 hours, little to no αSMA expression in the mesothelium suggests fibroblasts were not activated. However, αSMA+ve fibroblasts were detected at 6 hours suggesting an increased in fibroblast activity and recruitment to the mesothelium. At 24 hours (**Fig. 5c**), the blood clot seemed to have started lysing. Although discontinuous mesothelium was still observed, there were regions of the mesothelium that were slightly thicker than others. These thicker regions corresponded with a more intense staining for PSR but low expression of αSMA and Cx43.

At 48 hours (**Fig. 5d**), discontinuous mesothelium was less frequently observed and appeared thicker. These observations suggest that healing may have taken place. Consistent with this, thicker mesothelium regions corresponded with increased PSR staining and αSMA and Cx43 expression. At 72 hours (**Fig. 5e**), adhesions juxtaposing along the mesothelium were observed. These adhesions stained positively for PSR and were surrounded by mesothelium expressing high levels of αSMA and Cx43.

Discussion

Adhesion formation required the contact, abrasion, and clotted blood

A rat peritoneal strip *ex vivo* adhesion model detected visible adhesive bands when abraded peritoneal strips were sutured and subsequently cultured in the presence of clotted blood. [8] Here, we described a mouse peritoneal strip *ex vivo* adhesions model, with modifications made to the previously published experimental conditions and we show that adhesions can be reproducibly created. The key features of this mouse peritoneal strip *ex vivo* adhesions model were the use of gauze padding in tissue cassettes to prevent the unfolding of tissue strips (rather than sutures as used in the rat model) and the use of a standardised volume of clotted blood. Macroscopic adhesion bands were reproducibly observed after 72 hours. Adhesion formation was confirmed by histological assessment (H&E and PSR) and immunohistochemical staining (αSMA and Cx43).

Contact between the peritoneal surfaces is required for reproducible adhesion development. In pilot studies, we found suturing of the mouse peritoneal strips to be cumbersome, have the propensity to introduce additional grasping damage to the tissue, and the potential to introduce a foreign body reaction. As these factors could introduce variables to the model, we refined the approach to use a tissue histology cassette to prevent the abraded portions from unfolding while in culture. The cassette also helped to keep the strips in place during culture. The folded peritoneal strips were padded with sterile cotton gauze to increase contact between the opposing parietal surfaces. This simulated an

approximation of two injured surfaces commonly performed in post-surgical in vivo adhesion experimental models.[11-14] By improving the positional stability, we standardised the location and area of adhesion creation, which resulted in an increase in the baseline incidence and extent of adhesions created [10]. Adhesions were found in all the mouse peritoneal strips cultured in the presence of the gauze, but only 4 out of 7 strips cultured in the without the gauze. The adhesions observed were consistent with "Type 1B adhesions" in humans.[9] This aligns with clinical practices, as minimising contact between two opposing traumatised regions after surgery is an important step, and also justifies the requirement to use clinical barrier agents targeting adhesion formation.[15]

The model may simulate bleeding that occurs during surgery and create tissue trauma from manipulations during surgical procedures through the presence of clotted blood and abraded surfaces, respectively. No adhesion bands were observed when an abrasion injury was absent in either the rat or mouse models, suggesting that this trauma is a key contributor to adhesion formation. Additionally, no adhesions were formed when abraded strips were cultured without clotted blood, suggesting that this is also an essential factor in the development of adhesions. The essential role of blood is in line with current understanding, as the blood facilitates the initial adhesion of two surfaces during early adhesion formation by providing a source of fibrin and immune cells [10, 16]. This observation is also consistent with surgical practices, where it is important to achieve haemostasis to prevent/minimise adhesion development in the patient.[17] Previously, there was no standardisation of the amount of clotted blood placed between the strips in the rat model [8], which introduced potential variations. A standardised volume of 200 μL was used in this study, and larger volumes of clotted blood did not increase the quality of adhesion created (data not shown).

Differences between the rat and mouse ex vivo models included: propensity to form adhesions in sub-optimal conditions, timings of adhesion development and appearance of the adhesions. For example, although no adhesions were detected in our model when mouse peritoneal strips cultured in the absence of clotted blood after 72 hours, the rat model reported adhesions detected as early as 48 hours in strips cultured under the same conditions.[8] This difference suggests that rat tissues may have a higher propensity to form adhesions. The differences observed may be due to interspecies variation in fibrinolytic activity [18, 19, 10]. In the murine strips with abrasion and clotted blood, the adhesive bands were observed after being in culture for 72 hours and the majority of adhesions were thin and filmy, requiring gentle traction to disrupt. In contrast, the adhesive bands described in the rat model, were observed as early as 24 hours and noted to be obvious, "dense and opaque" with appreciable vascularity [8].

Validation of adhesion formation with microscopic assessment

As the majority of the adhesive bands were thin and filmy and lysed upon unfolding of the strip, we preserved the adhesions in their physiological state by processing intact strips and paraffin embedding them *en bloc* for histological assessment. As it can be challenging to pinpoint the exact location of adhesions microscopically without staining, in each case 5 tissue sections were sampled from 5

positions at 500 μm intervals along the peritoneal strip. The presence of adhesions was confirmed by the observation of eosinophilic matrices between juxtaposing mesothelium and collagen deposition (inferred from the positive staining of PSR in these matrices) [20] Additionally, surrounding mesothelium peripheral to the matrices expressed high levels of αSMA. This may suggest mesothelial-to-mesenchymal transition[21] and sub-mesothelial fibroblast activation to the adhesion phenotype.[5] Notably, regions with no adhesions were negative for these markers. This demonstrates that these expression patterns were unique to abrasion and blood clot treatment.

Interesting, the mesothelial layer (assessed by H&E) was found to thicken over time in the tissues where adhesions formed, similar to that described in a model of post-surgical adhesions [22]. This thickening of mesothelium resembles what is observed in human patients, suggesting our model closely mimics the human condition [23-25]. Conversely, in conditions that did not result in adhesions (conditions 2 and 4; Table 1), the mesothelium remained thin even at the later time points. Considering the discontinuous nature of the mesothelium due to abrasion reduced over time, this suggests that some degree of healing had occurred. However, unlike normal inflammatory events, little or no infiltration of immune cells were observed. This could have limited the magnitude of the adhesion created .[10]

Considerations for future use of this model

Having a mouse model available provides the additional advantage of supporting researchers with investigations using genetically modified transgenic murine strains and widely available commercial immunological reagents and/or assay kits designed for murine systems.

However, due to the larger size of the rat peritoneal cavity it may present a more "cost effective" approach for research, with approximately 6 strips obtained from each rat compared to 3 strips from each mouse. Nevertheless, 3 strips are sufficient for assessment of multiple treatments on tissue from one animal. Furthermore, paired dose-response comparisons and intra-animal paired comparisons for different test articles can be accomplished with multiple strips [8].

A major limitation of any ex vivo model is the lack of blood circulation. One of the early inflammatory responses to the inflicted injury of the peritoneum is characterised with the infiltration of the immune cells [22, 15]. Both resident and infiltrating cells release a cocktail of inflammatory mediators [15]. Many of these mediators have been shown to play a major role in adhesion formation [26]. One of earliest cytokines present in the injured peritoneal cavity is IL-1, which causes human peritoneal mesothelial cells to proliferate [27]. However, due to the nature of the model, only tissue resident immune cells and those that are found in the clotted blood were present. These contribute very little to inflammation in the model.

The incidence of adhesions may be reported when assessing the efficacy of test articles in the mouse model, as an "adhesion-free outcome" is a meaningful result [10]. However, as most adhesions were thin and filmy, this may make the model too "permissive" such that all test articles become effective. On the contrary, the adhesive bands generated in the rat model were described to be dense, opaque, and vascularised. Although the number of abrasions was standardised, it is difficult to optimally apply

consistent pressure during scalpel abrasion due to the flaccidity of the strip. This may account for the observed heterogeneity of adhesive bands created across the strip. Making microscopic assessment of adhesions and screening the efficacy of anti-adhesion agents challenging.

Interestingly, we observed that Cx43 protein levels were observed to be higher in regions where adhesions were present using this model (Figure 4e). This finding demonstrates that it is possible to study the development of adhesions, identify molecules of interest and test therapeutics. As far as we are aware this is the first report that Cx43 has a role in adhesion formation.

Conclusion

We report the use of mouse peritoneal strips in creating an *ex vivo* adhesion model. Through the use of gauze padding in tissue cassettes and standardised volume of clotted blood we show that abraded surfaces reproducibly develop adhesion bands at 72 hours. Adhesions were visible macroscopically and confirmed by microscopic assessment. We also found a novel association of Cx43 in adhesion formation.

Declarations

Funding

Conflicts of interest

None of the authors declare any conflict of interest.

Ethics approval

All animal procedures were performed in accordance with the animal protocols approved by the Institutional Animal Care and Use Committee of the Animal Research Facility of Nanyang Technological University, protocol number A0372.

Availability of data and material

All original data and samples are held by DL. Becker at Lee Kong Chian School of Medicine, Nanyang Technological University Singapore.

Code availability

Not applicable

Authors contributions

Jia Wang Chua made substantial contributions to the conception and design of the work, acquisition of the data and analysis, and interpretation of data.

David L. Becker made substantial contributions to the conception and design of the work, analysis, and interpretation of data.

Anthony RJ. Philips made substantial contributions to the conception and design of the work and interpretation of data.

Leigh Madden made substantial contributions to the conception and design of the work and interpretation of data.

Sophia Lim was involved in revising it critically for important intellectual content.

All authors were involved in drafting the work or revising it critically for important intellectual content and have approved final version of work to be published.

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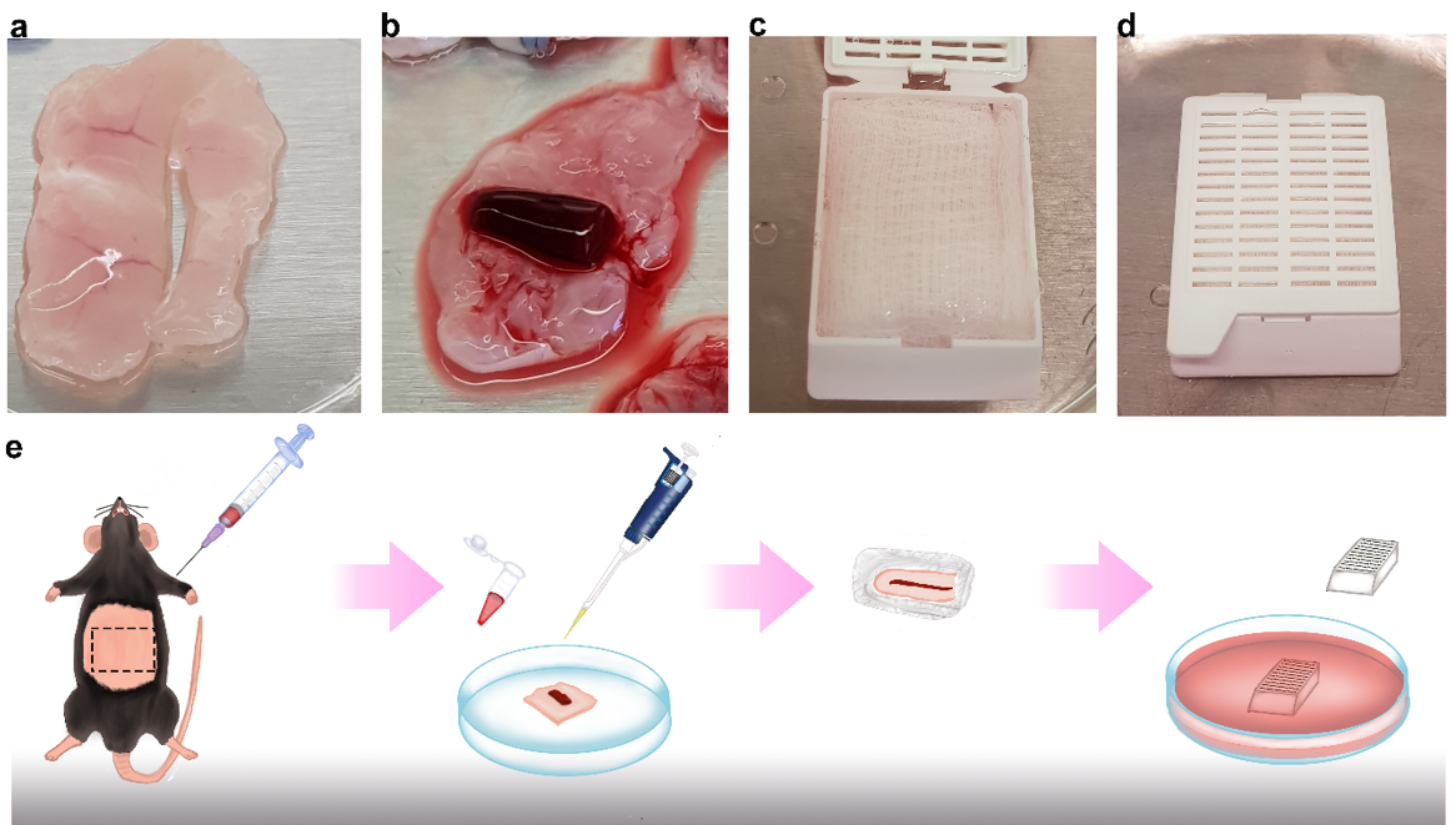
Figures

Figure 1

Steps for inducing ex vivo adhesions formation (a) Peritoneal strips were harvested and trimmed. Depending on the condition explored (Table 2), each strip was abraded and/or had a clotted blood placed on the peritoneal surface. (b) Example of condition 1, where a blood clot is placed on an abraded portion of the strip. (c) Strips were then folded in half with peritoneal surfaces facing each other and padded with cotton gauze before being placed in a tissue histology cassette. (d) The cassettes were then placed in a 100-mm cell culture dish with media and incubated at 37°C and 5% CO_2 for up to 72 hours. (e) Schematic diagram representing steps in ex vivo adhesion model.
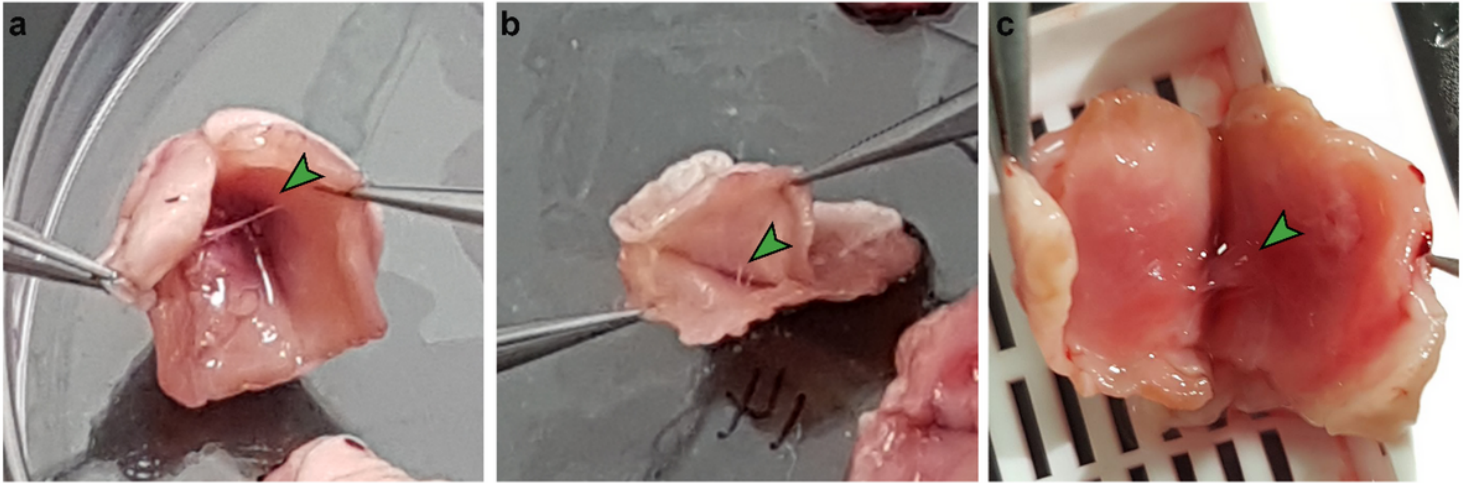

Figure 2

Examples of ex vivo adhesions formed in peritoneal strips cultured Peritoneal strips cultured under different conditions were inspected for the presence of adhesion bands at 72 h post-induction of ex vivo adhesion formation. Cultures of peritoneal strips under condition 1 (n=5, 2 experimental repeats), in which clotted blood was placed between abraded surfaces, resulted in the development of reproducible adhesion bands (green arrowheads). Examples of peritoneal strips that were observed to have one (a) or two (b) thin filmy adhesion bands that may be broken by gentle traction. (c) Example of a peritoneal strip observed to have a thick adhesion band requiring blunt dissection to be broken.
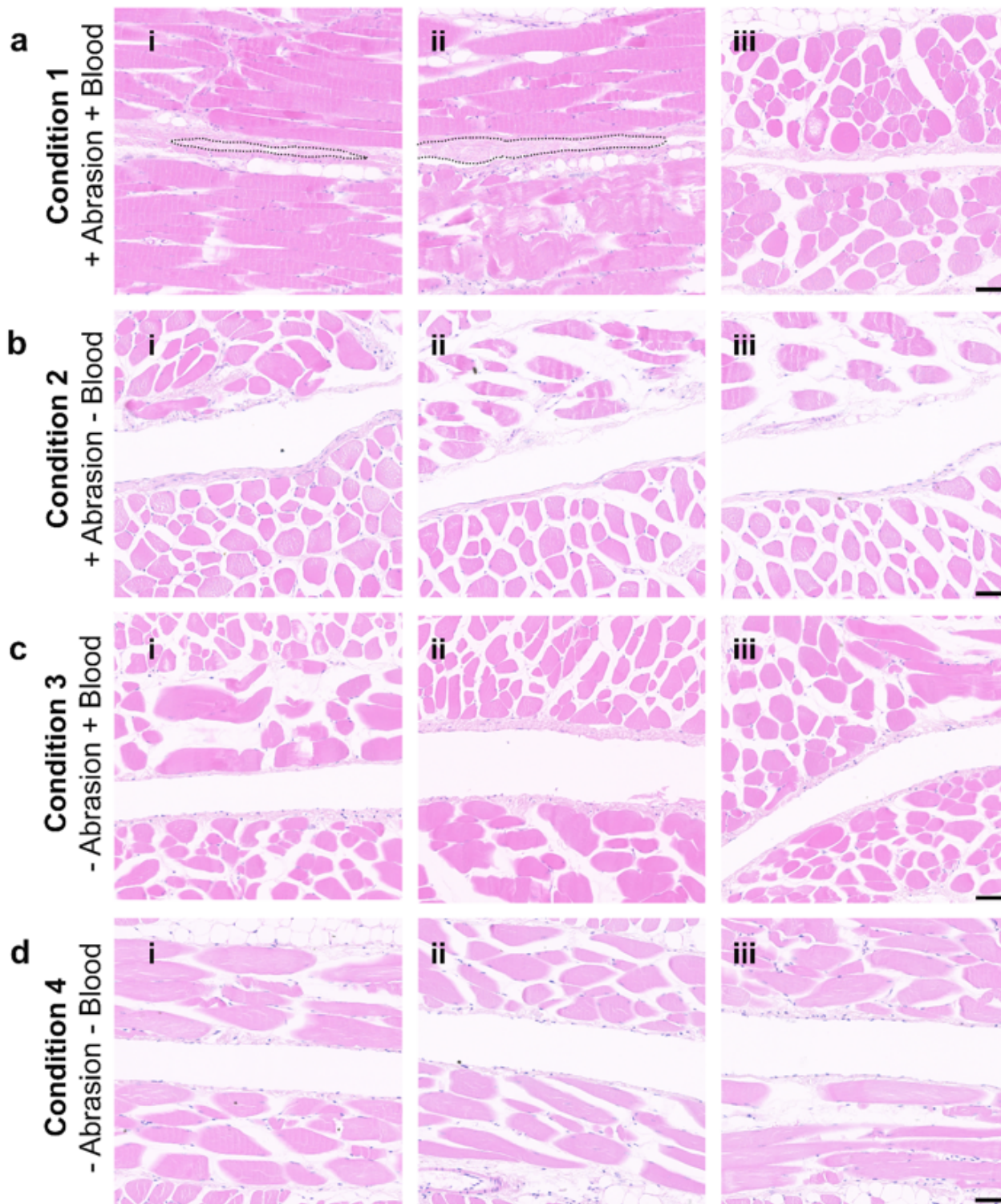
Figure 3

H&E assessment of cultured peritoneal strips cultured under different conditions Three representative regions were selected for each peritoneal strip cultured under different conditions (n=5, each). (a) Under condition 1, where abrasion and clotted blood were present, the mesothelium appeared to be mostly continuous. Some regions along the strip had thickening of mesothelium and in certain regions of juxtaposed mesothelium, areas of adhesions were observed (marked with dotted lines in i and ii). (b)

Under condition 2, where abrasion was present but clotted blood was absent, some regions of mesothelium had poor integrity (i) or discontinuous mesothelium (ii and iii). (c) Under condition 3, where abrasion is absent but clotted blood was present, thin mesothelium was observed in most regions (i and iii) except a few regions where mesothelium was thicker (ii). (d) Under condition 4, where both abrasion and clotted blood were absent, thin continuous mesothelium was observed across the entire strip (i to iii). Scale bar at 50 µm.
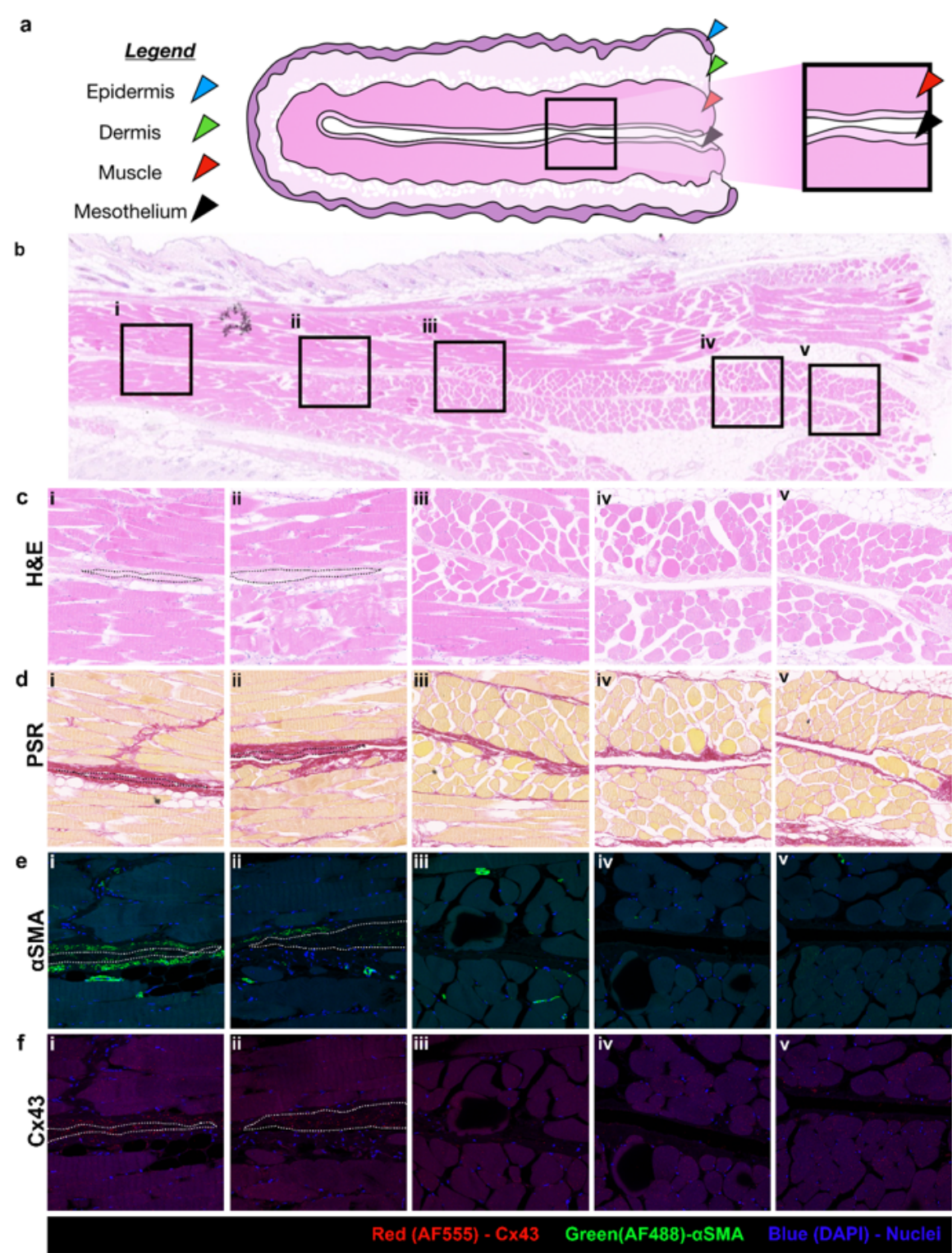

Figure 4

Microscopic assessment of peritoneal strips that were cultured with a placement of blood clot between abraded surfaces. (a) Schematic diagram showing the different layers of the cultured peritoneal strip. Boxed region illustrates an example of region of interest that focuses on the juxtaposing mesothelium and underlying muscle. (b) Representative stitched image of H&E-stained peritoneal strip. Five random regions (as indicated by black boxes) along a representative strip incubated under condition 1 (n=5) were selected and assessed with different histological (H&E and PSR) or immunohistochemical stains (αSMA and Cx43). (c) Some eosinophilic matrix (dotted lines) were only observed in certain regions (i and ii) between juxtaposing mesothelium along the strip. These regions were likely to be areas where adhesions have developed as indicated from positive staining of PSR (d) and αSMA (green) (e). These regions were also found to have higher Cx43 protein levels (red) (f). Scale bar at 50 μm.
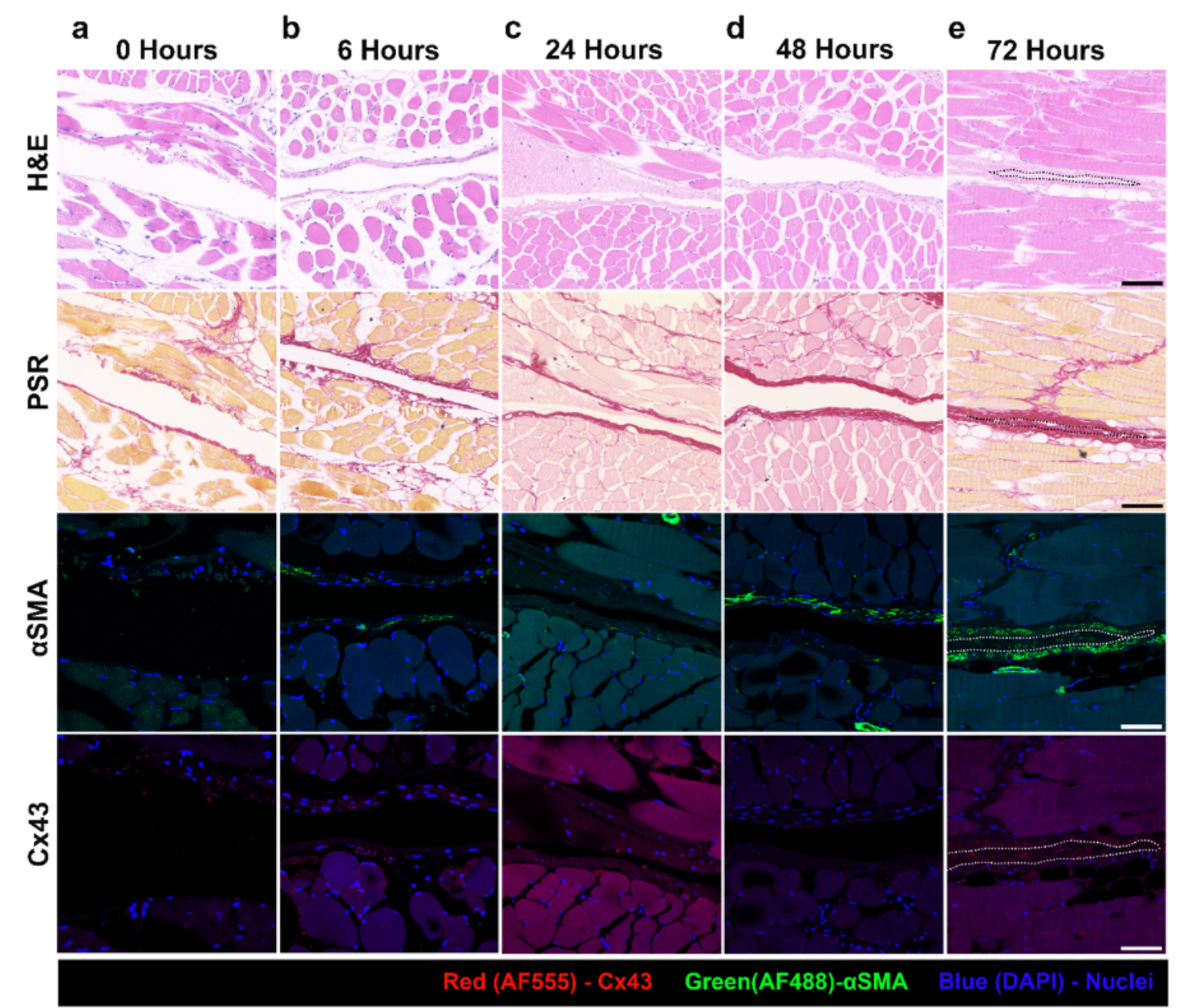

Figure 5

Microscopic assessment of peritoneal strips cultured under condition 1 over 72 hours Abraded peritoneal strips cultured in the presence of blood (condition 1), resulting in reproducible development of adhesion bands, were harvested at progressive time points. The strips of each time point (n=5) were assessed with different histological (H&E (a) and PSR (b)) or immunohistochemical stains (αSMA, in green (c) and Cx43, in red (d)). One representative region along the strip was selected to provide a general overview of the development over time. Dotted lines indicate areas where adhesions were detected. Magnification used, Scale bar at 50 μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx