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Generation and Evaluation of Novel Stromal Cell-Containing Tissue Engineered Artificial Stromas for the Surgical Repair of Abdominal Defects

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Repair of abdominal wall defects is one of the major clinical challenges in abdominal surgery. Most biomaterials are associated to infection and severe complications, making necessary safer and more biocompatible approaches. In the present work, the adequate mechanical properties of synthetic polymer meshes with tissue-engineered matrices containing stromal mesenchymal cells is combined to generate a novel cell-containing tissue-like artificial stroma (SCTLAS) for use in abdominal wall repair. SCTLAS consisting on fibrin-agarose hydrogels seeded with stromal cells and reinforced with commercial surgical meshes (SM) are evaluated in vitro and in vivo in animal models of abdominal wall defect. Inflammatory cells, collagen, and extracellular matrix (ECM) components are analyzed and compared with grafted SM. Use of SCTLAS results in less inflammation and less fibrosis than SM, with most ECM components being very similar to control abdominal wall tissues. Cell migration and ECM remodeling within SCTLAS is comparable to control tissues. The use of SCTLAS could contribute to reduce the side-effects associated to currently available SM and regenerated tissues are more similar to control abdominal wall tissues. Bioengineered SCTLAS could contribute to a safer treatment of abdominal wall defects with higher biocompatibility than currently available SM.

1. Introduction

Tensionless repair of abdominal wall defects is one of the major clinical challenges in abdominal surgery, since reconstruction under tension is associated with surgical failure and high recurrence rate.[1] In cases in which native tissue is damaged or absent, it is necessary to use prosthetic materials allowing an effective abdominal wall closure. In fact, the use of prosthetic surgical meshes – SM – is widely accepted in routinely performed surgeries including eventration and hernia repair and other abdominal wall procedures in which the use of prosthetic materials has been well established.[2,3] The use of highly resistant non-degradable synthetic SM materials demonstrated to prevent hernia recurrence in controlled clinical trials.[4] Despite their efficiency, most commonly available synthetic SM materials are prone to infection and can erode into the bowel, leading to entero-cutaneous fistula formation and other severe complications,[5] making necessary the search of safer and more biocompatible approaches.[6]

In order to minimize the incidence of complications associated to the use of synthetic SM, other types of biological materials were developed from human or animal tissues, although a recent systematic review of their efficacy concluded that biological SM are not objectively superior to synthetic materials.[5] In contrast to synthetic materials, biological SM are typically more biodegradable and offer higher biocompatibility, although their biomechanical properties use to be suboptimal. In this milieu, the recent development of tissue engineering techniques allows the construction of highly compatible bioengineered tissues using stem cells, biomaterials, and other factors.[7] The use of stem cells has been extensively used due to high regenerative potential due to their proliferation capability, the production of anti-inflammatory molecules, and migratory potential of these cells toward the injury site.[8] All these stem cells properties make them suitable candidates for tissue engineering approaches. In this context, our research group was able to generate several models of biomimetic artificial

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tissues such as the human cornea,[9] skin,[10] and oral mucosa,[11] some of which have already been transferred to the clinical setting. In all these cases, natural fibrin-agarose hydrogels were combined with stromal adult stem or stromal cells to reproduce the histological structure of native connective tissues by generating a bioengineered stroma. Other researchers described the use of several types of meshes and devices generated by tissue engineering using recellularized dermal acellular matrices[12] and other materials such as silk fibroin,[13] collagen, and intestinal submucosa[14] previously seeded with cells. All these bioengineered substitutes offer high biocompatibility as compared to synthetic materials. However, their biomechanical properties use to be rather poor as compared to synthetic SM.

Since ideal surgical prosthetic implants should achieve the most effective repair, including wound healing and mechanical outcome,[15] in the present work we combined the advantages of tissue substitutes generated by tissue engineering with the biomechanical properties of two of the most commonly used synthetic polymeric SM in order to generate a novel stromal cell (SC)-containing tissue-like artificial stroma (SCTLAS) consisting of a fibrin-agarose hydrogel seeded with mesenchymal SC reinforced with a SM. Then, we analyzed these structures both in vitro and in vivo in animal models.

2. Experimental Section

2.1. Isolation and Culturing of Mesenchymal SC

Forty Wistar rats were used in the present work. Animals were deeply anesthetized and biopsies of the dorsal skin were obtained. Cell isolation and culture were performed as previously described.[16] Briefly, tissues were trimmed and washed in phosphate-buffered saline and digested in 2 mg mL^{-1} type I collagenase (Gibco, Karlsruhe, Germany) at 37° C to isolate the tissue SC. Isolated SC were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, antibiotics and antimycotics (100 U mL^{-1} penicillin G, 100 mg mL^{-1} streptomycin, and 0.25 mg mL^{-1} amphoterycin B, Sigma–Aldrich) using standard cell culture conditions.

This study was performed with the approval of the Institutional Animal Care and Use Committee of Granada (ref. PI141343) and in compliance with international standards for animal care. Animals were provided and maintained in the Experimental Unit of the University Hospital Virgen de las Nieves in Granada (Spain). They were kept in individual cages in a temperature-controlled room (21 ± 1 °C), on a 12 h light/dark cycle with ad libitum access to tap water and standard rat chow.

2.2. Generation of Bioengineered Tissue-Like Artificial Stromas (SCTLAS)

Rat skin SC were immersed in fibrin-agarose hydrogels (FA) as previously described.[17] Briefly, human plasma – as a source of fibrin – was mixed with 0.1% agarose, tranexamic acid, and 250 000 cultured SC, and calcium chloride was used to polymerize the hydrogel. To generate SCTLAS with improved mechanical properties, a commercial SM was immersed within the FA before polymerization. Two different SM were used: partially absorbable multifilament polypropylene and polyglactine 910 (PP/PG) meshes (Vypro II, Ethicon, Hamburg, Germany) and non-absorbable polypropylene (PP) meshes (Prolene, Ethicon, Sommerville, NJ, USA). SCTLAS were maintained in vitro using standard culture conditions up to 35 days, when histological and histochemical analysis were performed.

2.3. Study Groups and Surgical Procedures

The following study groups were established in the present work:

1) FA group: FA gels consisting in fibrin-agarose with SC. These samples were analyzed in vitro.
2) SM group ($n = 15$): PP (PP-SM) or PP/PG (PP/PG-SM) meshes grafted in vivo in animal models (control group of grafted surgical mesh resembling the clinical situation).
3) SCTLAS group ($n = 15$): SCTLAS with PP meshes within (PP-SCTLAS) or PP/PG meshes within (PP/PG-SCTLAS). These samples were analyzed in vitro and grafted in vivo in animal models.
4) Native control group ($n = 10$): Stromal tissue corresponding to the connective tissue allocated at the midline of the rat abdominal wall was used as control of normal native tissue.

All in vitro samples corresponded to samples kept in culture for 35 days. In vivo samples were grafted in the abdominal wall of laboratory rats in which an abdominal wall defect was surgically generated (Figure S6, Supporting Information). In brief, animals were deeply anesthetized by intraperitoneal injection of a mixture of ketamine (Imalgene 1000®, 0.15 mg g^{-1} body weight) and acepromazin (Calmo-Neosan®, 0.001 mg g^{-1} body weight). Then, a median laparotomy of approximately 2 cm length was generated in each animal to resemble a full-thickness midline abdominal defect affecting all tissue layers. Then, a SM (PP-SM or PP/PG-SM) or SCTLAS (PP-SCTLAS or PP/PG-SCTLAS) was surgically implanted and sutured at both sides of the defect using absorbable suture material. Finally, the abdominal injury was repaired by suturing the subcutaneous tissue using absorbable material and the skin using non-absorbable silk stitches. After surgery and wound closure, all rats were kept in individual cages and received metamizole in the drink water as analgesia for 7 days (100 mg metamizole per kg body weight each 24 h). Animals were euthanized after 14 or 28 days of the surgical procedure for histological and histochemical analyses.

2.4. Histological and Histochemical Analyses

In vitro and in vivo samples were fixed in 10% buffered formalin, embedded in paraffin, and 5 μm-thick sections were obtained. Sections were stained with hematoxylin and eosin and histologically analyzed using a Nikon Eclipse 90i light microscope. To determine the presence of inflammatory cells in each in vivo sample of the SM and SCTLAS groups, five images were obtained from each animal using 200× magnification, and the

region-of-interest (ROI) of each sample – corresponding to stromal tissue surrounding the filaments of the mesh – was analyzed. Quantification was carried out by using ImageJ software (McBiophotonics, Ontario, Canada).

Histochemical analysis of the main fibrillar and non-fibrillar components of the extracellular matrix (ECM) of the abdominal wall stromal tissue was performed as previously described.[18] Briefly, mature fibrillar collagen was assessed by picrosirius histochemical methods using a Sirius red F3B working solution for 30 min and Harris's Hematoxylin counterstaining. Collagen intensity in the ROI around the PP or PP/PG filaments was quantified by using ImageJ software. Fiber orientation was analyzed by polarized light microscopy. To evaluate reticular fibers, tissue sections were stained with the Gomori's reticulin metal reduction histochemical method using 1% potassium permanganate, 2% sodium metabisulphite solution, and sensibilization with 2% iron alum. Then, samples were incubated in ammoniac silver and 20% formaldehyde. For elastic fibers, the orcein histochemical method was used. ECM glycoproteins were detected by the periodic acid-Schiff histochemical method (PAS) counterstained with Harris's hematoxylin for 1 min. Finally, samples were incubated in alcian blue histochemical solution for 30 min and counterstained with nuclear fast red solution for proteoglycans detection.

Immunofluorescence analysis of type I-collagen, cortactin, and MMP-14 expression was carried out on formaldehyde-fixed, paraffin-embedded tissue sections using standard procedures. Briefly, paraffin was removed from the tissue sections using xylene, and endogenous peroxidase was quenched in 3% H_2O_2. Then, we used 0.01 M citrate buffer (pH 6.0) (Dako, Glostrup, Denmark) at 95 °C for 25 min for antigen retrieval. Incubation with the primary antibodies was performed for overnight at 4 °C using anti-type I-collagen (1:200, Acris, Rockville, MD, USA), anti-cortactin (1:100, Abcam, Cambridge, MA, USA), and anti-MMP-14 (1:500, Abcam). Subsequently, secondary Cy3-conjugated anti-rabbit antibody (Sigma–Aldrich, Steinheim, Germany) was used at 1:500 dilution. Tissue sections were counterstained with DAPI mounting medium (Vector Laboratories, Burlingame, CA) and mounted on coverslips for optical evaluation using a fluorescence light microscope (Nikon Eclipse). Characterization of the SC included in the biomaterial was carried out by immunohistochemistry for the vimentin, CD90 and CD105 markers. Briefly, samples were prepared as described for immunofluorescence, and primary anti-vimentin (1:200, Sigma–Aldrich), anti-CD90 (1:50, Novus Biological, Cambridge, UK), and anti-CD105 (1:200, Vector Laboratories, Burlingame, CA) antibodies were incubated overnight. Then, peroxidase-labeled secondary antibodies (Vector Laboratories) were used and the signal was detected by incubation in a DAB solution (Vector Laboratories). To characterize the inflammatory cells present in the biomaterials grafted in vivo, we used the same immunohistochemical procedure by using monoclonal anti-macrophage antibodies (1:50, Abcam).

2.5. Scanning Electron Microscopy

In vitro SCTLAS included in this study were processed for scanning electron microscopy (SEM) as previously described.[19] Briefly, samples were rinsed in distilled water, dried on paper and immediately immersed in liquid nitrogen and kept at −196 °C until processing. To sublimate all water from the samples, they were freeze-dried in an Emitech K775 high-vacuum system (Emitech, Walford, UK) for 17 h. The samples were left in the freeze-dryer and allowed to slowly return to room temperature to prevent condensation of atmospheric water on the surface. Finally, samples were mounted and covered with gold in an argon atmosphere at $p = 10^{-5}$ mbar for 30 s. Samples were analyzed in an FEI Quanta 200 environmental SEM (FEI Europe, Eindhoven, Netherlands).

2.6. Statistical Analysis

Descriptive analysis and U Mann–Whitney test were performed using SPSS 17 (SPSS Inc, Chicago, IL, USA) to compare number of inflammatory cells and expression of collagen between SM and SCTLAS. Kendall tau *b* test was carried out to correlate number of inflammatory cells and expression of collagen with the presence/absence of SCTLAS. Level of significance was set at $p < 0.05$ for all tests.

3. Results

3.1. Surgical Procedure and Histological Analysis

The method described in this work allowed us to generate novel bioengineered tissues useful for the repair of abdominal wall defects. All animals tolerated the surgical procedure and survived to the implant of the different SM and SCTLAS materials. No infections, necrosis, or severe complications and no signs of digestive obstruction were detected.

Histological analysis of the FA, PP-SCTLAS, and PP/PG-SCTLAS developed in laboratory and kept in vitro revealed an abundant fibrin-agarose extracellular material with a high number of cells with a typical long-shaped morphology as an indicator of cell viability (**Figure 1**). SC characterization revealed positive signal for vimentin and negative expression for CD90 and CD105 (data not shown). In addition, SEM analysis suggested a proper integration of the different meshes with the fibrin-agarose biomaterial (Figure 1).

Once implanted in vivo, a proper biointegration of both types of SCTLAS was found and a number of host cells were able to migrate and colonize grafted SCTLAS. In fact, histological analysis showed that the grafted SCTLAS were rapidly bioremodeled by the host tissue, with the formation of newly formed blood vessels, especially after 28 days (black arrows in Figure 1). No microscopic signs of necrosis, infection or tumorigenesis were detected.

In contrast, animals grafted with both types of SM showed an intense inflammatory reaction surrounding the fibers of the PP-SM and PP/PG-SM. Immunohistochemical analysis demonstrated that most inflammatory cells were macrophages (Figure 1). Results corresponding to the quantification of inflammatory cells after 14 and 28 days post-implantation of SM and SCTLAS are shown in **Figure 2**. The presence of SCTLAS was related with less accumulation of inflammatory cells when

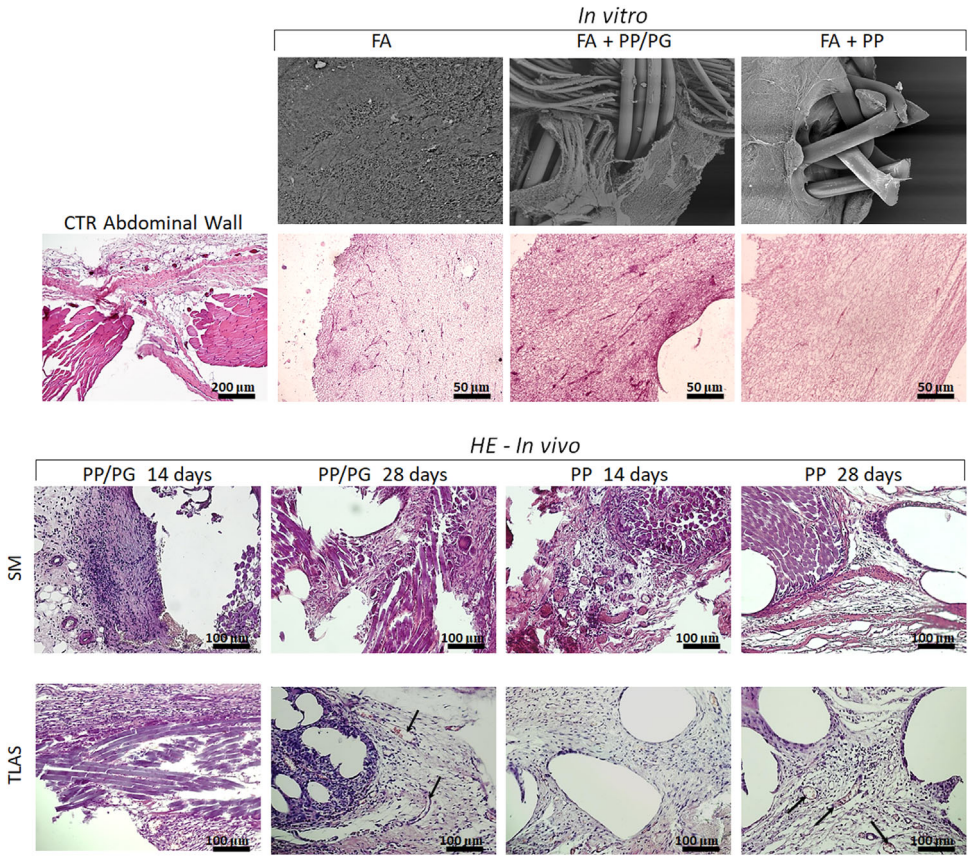
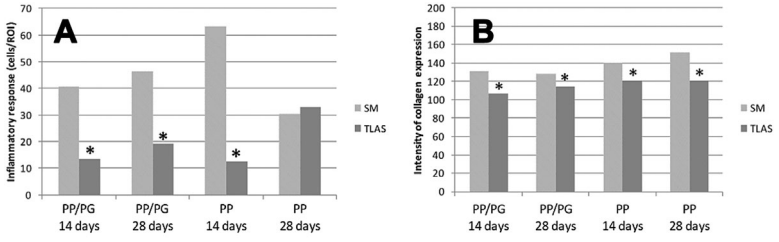


Figure 1. Scanning electron microscopic images and histological analysis of the different samples analyzed in this work using hematoxylin and eosin staining. Black arrows point newly formed blood vessels. CTR abdominal wall, native control corresponding to the connective tissue allocated at the midline of the rat abdominal wall; FA, fibrin-agarose hydrogels with SC; SM, surgical mesh; SCTLAS, tissue-like artificial stroma; PP, polypropylene; PP/PG, polypropylene-polyglactin 910.

compared to the SM. SM group animals showed more than twofold inflammatory cells (45.13 ± 26.22 cells/ROI) as compared with those whose abdominal defects were treated by SCTLAS implantation (19.57 ± 7.83 cells/ROI) ($p < 0.001$). Furthermore, a significant correlation was detected between the study group (SM group or SCTLAS group) and the number of inflammatory cells ($\tau = 0.554$, $p < 0.001$). For each time period (14 and 28 days) and each mesh type (PP or PP/PG), local



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		INFLAMMATORY CELLS			
		PP/PG		PP	
		14 days	28 days	14 days	PP 28 days
SM	Average	40.50	46.40	63.20	30.43
	SD	23.86	31.15	25.14	20.98
TLAS	Average	13.50	19.33	12.43	33.00
	SD	4.04	5.05	6.40	9.90
		FIBROSIS			
SM	Average	130.93	128.83	140.43	151.60
	SD	32.43	30.09	23.29	28.07
TLAS	Average	106.90	114.73	121.07	120.80
	SD	12.35	27.95	19.16	14.78

Figure 2. Quantification of inflammatory cells and fibrotic reaction in samples grafted in vivo for 14 and 28 days. Top panels: histograms representing the number of inflammatory cells per region of interest (ROI) (A) and the intensity of collagen fibrotic reaction (B) in the surgical mesh groups (SM) and the tissue-like artificial stroma groups (SCTLAS). Bottom panel (C) shows the average and standard deviation (SD) values corresponding to the same samples.

inflammatory response was significantly higher when the SM was grafted alone, except for PP meshes after 28 days ($p > 0.05$).

3.2. Analysis of Fibrillar Components of the Extracellular Matrix

Synthesis of fibrillar proteins has been assessed after implantation of SCTLAS abdominal wall substitutes. First, immunofluorescence analysis of type I-collagen showed that cells in the SCTLAS were capable to express some type I-collagen fibers after 35 days of in vivo culture previous to implantation (Figure S1, Supporting Information and **Table 1**). Then, expression of type I-collagen was more intense after in vivo implantation in all groups. Control SM showed higher expression of collagen near the mesh filaments. No differences were found between PP and PP/PG.

Formation of mature extracellular collagen fibers was evaluated by picrosirius staining (**Figure 3** and Table 1). Our results showed that mature collagen fibers were negative in all in vitro samples previous to implantation and positive and well oriented in control abdominal wall. However, after in vivo host implantation during 14 and 28 days, some collagen fibers were detected inside the artificial tissues in SCTLAS groups and surrounding the polymer filaments in the SM group. In fact, collagen fibers tended to form a dense structure that encapsulates the mesh fibers in the SM group. Interestingly, quantification of the intensity of collagen expression revealed that the amount of collagen was higher in SM (137.95 ± 29.72) than SCTLAS (115.88 ± 20.10) ($p < 0.001$) as determined by Picrosirius staining (Figure 2). In addition, the statistical analysis showed higher intensity of collagen in PP-SCTLAS as compared to PP/PG-SCTLAS ($p < 0.001$). On the other hand, analysis of collagen fibers by using polarized light showed that the amount of mature, well-oriented fibers was higher in SM than in SCTLAS groups (Figure 3B).

When elastic fibers were stained in the different sample, we found that these fibrillar components were absent from FA and SCTLAS samples kept in vitro, whereas control abdominal wall was highly positive. Implantation of SM resulted in positive expression of elastic fibers around the edges of the meshes, while these extracellular fibers were negative in SCTLAS substitutes implanted in vivo for 14 and 28 days (Figure S2, Supporting Information and Table 1). Finally, detection of reticular fibers showed that all samples, including native abdominal wall, were negative (Table 1).

3.3. Analysis of Non-Fibrillar Components of the Extracellular Matrix

ECM glycoprotein expression was evaluated by periodic acid-Schiff (PAS) staining. As shown in Figure S3, Supporting Information and Table 1, FA and SCTLAS cultured in vitro were negative for these ECM components. However, SCTLAS and SM grafted in vivo showed a mild, diffuse, and homogeneous glycoprotein pattern, showing very similar expression to untreated control abdominal wall tissues.

The expression of proteoglycans in ECM, as determined by alcian blue staining, also showed negative signal in all tissues kept in vitro, while control abdominal wall was strongly positive (Figure S4, Supporting Information and Table 1). However, SM grafted in vivo showed positive signal for these non-fibrillar ECM components, with 28 days PP-SCTLAS samples being highly positive. In vivo SCTLAS samples were very heterogeneous and showed maximum signal also for 28 days PP-SCTLAS samples.

3.4. Analysis of Cell Migration and ECM Remodeling

Evaluation of cell migration assessed by cortactin immunofluorescence (**Figure 4**A and Table 1) reported that some SC in SCTLAS showed variable expression of cortactin before implantation, being higher in PP-SCTLAS mesh than in PP/PG-SCTLAS, as well as control tissues, revealing that these cells maintained migration capabilities through the ECM in vitro.

Table 1. Semiquantitative analysis of ECM components in the different samples analyzed in this work.

	In vitro			In vivo (SM)					In vivo (TLAS)			
					PP/PG		PP		PP/PG		PP	
	FA	PP/PG	PP	CTR AW	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
Type-I collagen	+	+	+	+++	++	++	++	+++	++	++	++	++
Picrosirius	Ø	Ø	Ø	+++	+++	+++	+++	+++	+++	++	++	+++
Orcein	Ø	Ø	Ø	+++	++	+	+	++	Ø	Ø	Ø	Ø
Reticulin	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
PAS	Ø	Ø	Ø	+	+	+	+	+	+	+	+	+
Alcian blue	Ø	Ø	Ø	+++	++	++	++	+++	++	++	++	+++
Cortactin	Ø	+	++	+	++	+++	+++	++	+	++	++	+
MMP14	+	+	+	+	+++	++	+++	++	+	++	+	+

FA, fibrin-agarose hydrogels with SC; SM, surgical mesh; SCTLAS, tissue-like artificial stroma; PP, polypropylene; PP/PG, polypropylene-polyglactin 910; CTR AW, native control corresponding to the connective tissue allocated at the midline of the rat abdominal wall. Expression of each components has been determined as Ø (negative expression), + (mild expression), ++ (medium expression), and +++ (intense expression).

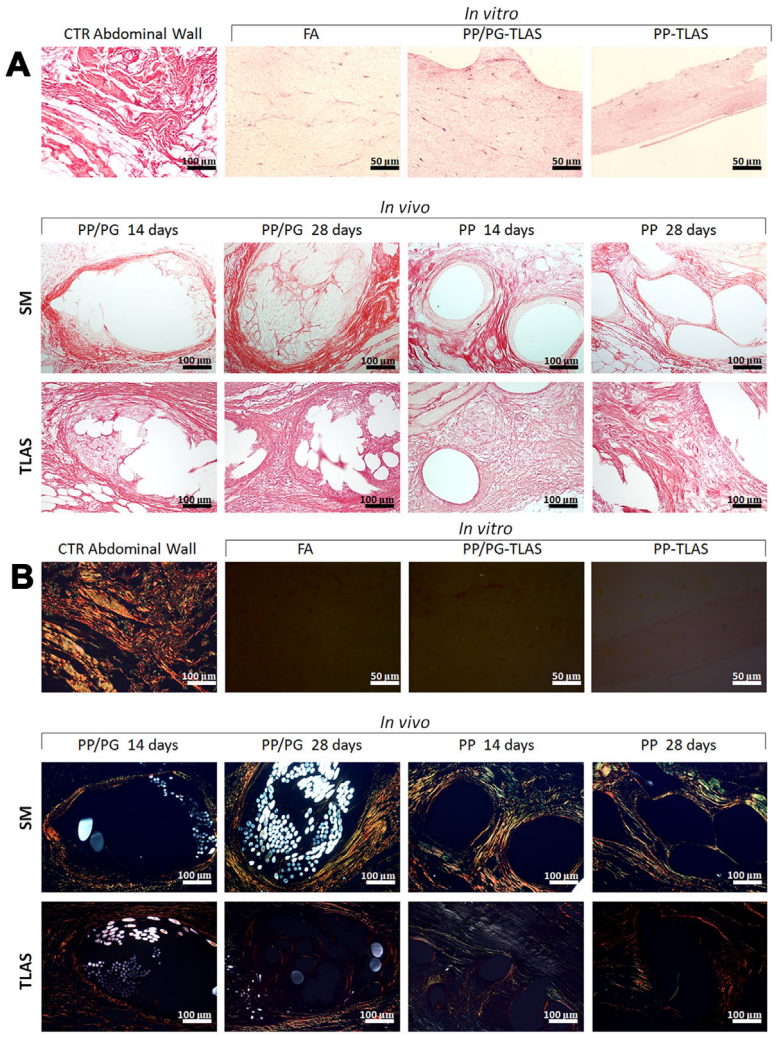

Figure 3. Histochemical analysis of collagen expression in the different samples analyzed in this work using picrosirius staining method under light (panel A) and polarized light (panel B). CTR abdominal wall, native control corresponding to the connective tissue allocated at the midline of the rat abdominal wall; FA, fibrin-agarose hydrogels with SC; SM, surgical mesh; SCTLAS, tissue-like artificial stroma; PP, polypropylene; PP/PG, polypropylene-polyglactin 910.

Control groups in which the SM was surgically grafted showed high or very high expression of cortactin, revealing active cell migration, especially at the periphery of the surgical fibers in PP/PG-SM after 28 days and PP-SM after 14 days. Regarding the behavior of SCTLAS implanted in vivo, results showed similar findings as compared to SM groups, but signal intensity tended to be lower.

Matrix metalloproteinase-14 (MMP-14) was also evaluated by immunofluorescence to evaluate ECM remodeling (Figure 4B and Table 1). Our results first showed that control abdominal wall connective tissue was virtually negative, as it was also the case of FA constructs kept in vitro. However, the presence of a surgical mesh induced some MMP-14 expression in SCTLAS samples kept in vitro, with no relevant differences between PP-SCTLAS and PP/PGA-SCTLAS. SM grafted in vivo showed high expression of MMP-14, especially after 14 days for both types of surgical materials (PP-SM and PP/PG-SM) and tended to decrease after 28 days. After implantation, SCTLAS substitutes revealed higher concentration of MMP-14 as compared to in vitro samples, suggesting that host stimulus could induce some ECM remodeling by fibroblast activation. MMP-14 expression was higher in SM when compared with SCTLAS.

4. Discussion

Reabsorbable and permanent surgical meshes are extensively used in abdominal surgery.[1,2] Although most available materials are safe and biocompatible, there are common drawbacks associated to the use of inert surgical meshes including infection, fibrosis, inflammatory reaction, and foreign body reaction.[20,21] For this reason, research in this field should be focused on the development of novel more biocompatible devices able to support and sustain the biomechanical requirements of clinical use in the abdominal wall. In this milieu, in the

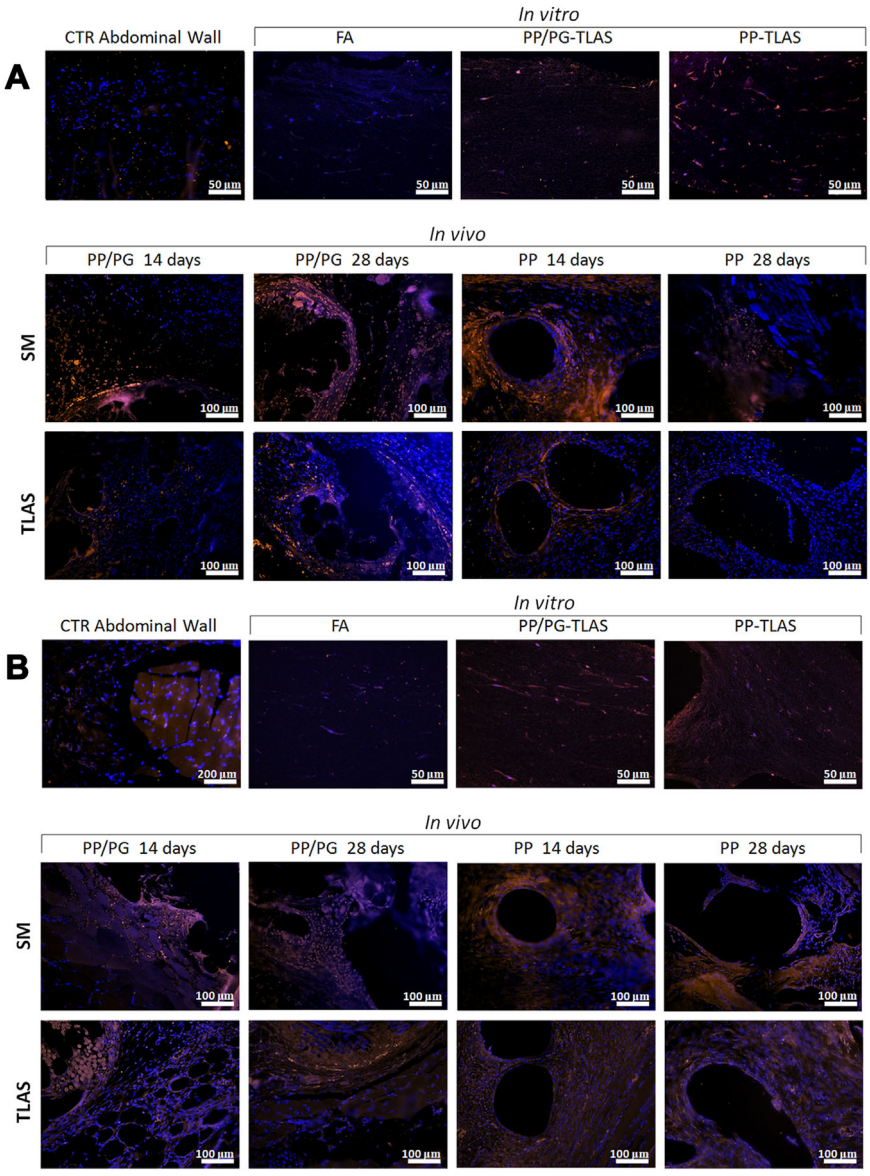

Figure 4. Immunofluorescence analysis of the different samples analyzed in this work using anti-cortactin (panel A) and anti-MMP-14 antibodies (panel B). CTR abdominal wall, native control corresponding to the connective tissue allocated at the midline of the rat abdominal wall; FA, fibrin-agarose hydrogels with SC; SM, surgical mesh; SCTLAS, tissue-like artificial stroma; PP, polypropylene; PP/PG, polypropylene-polyglactin 910.

present work we combined the advantages of tissue engineering and surgical meshes to develop a novel tissue-like artificial stroma containing SC, natural biomaterials based on fibrin-agarose and surgical meshes. This approach should allow us to obtain a highly biocompatible yet resistant graft able to exert the biocompatible properties of bioengineered tissues and the handling and suturing properties of synthetic surgical meshes.

One of the main advantages of the approach used in the present work is the use of highly biocompatible and biodegradable biomaterials combined with surgical meshes. In fact, fibrin-agarose biomaterials demonstrated to be safe and biocompatible in relevant animal models.[10,22] In addition, several previous works demonstrated that fibrin-agarose biomaterials could have appropriate biomechanical properties allowing future clinical use.[23,24] However, these biomaterials had not been associated to surgical meshes before.

Our results first showed that development of a joined structure combining a surgical mesh and a cellular hydrogel to obtain a SCTLAS was feasible in the laboratory by using simple methods and techniques based on cell culture and tissue engineering protocols. This straightforward procedure can be scalable and translated to GMP facilities for clinical use. In addition, SCTLAS were easily handled and allowed in vivo grafting and suturing due to the presence of a surgical mesh within the hydrogel.

Once grafted in vivo, our results showed that SCTLAS were properly integrated in the host abdominal wall tissues with no signs of rejection, infection or other complications, thus

suggesting that the bioengineered tissues were highly biocompatible, whereas control SM displayed some signs of inflammatory reaction mainly consisting of macrophage cells. Previous works demonstrated that synthetic SM may exert immediate and chronic inflammatory responses after in vivo implantation, although these responses depend on the properties of each individual mesh.[15,25–27] In fact, a recent report suggests that the in vivo use of biomaterials could be associated to a foreign body reaction with several phases and stages that may include a chronic inflammation phase with the presence of abundant macrophages.[27,28] In agreement with this, our analyses showed that SM grafting was associated to an inflammatory reaction with the presence of abundant macrophages. However, our results showed that the use of novel SCTLAS was associated to a lower amount of macrophages after 28 days, suggesting that SCTLAS could decrease chronic inflammation as compared to surgical meshes.[29] Further analyses should be performed to characterize in deep the specific types of macrophages present in each study group – M1 or M2 macrophages – and the eventual role or other inflammatory cells. A hypothesized predominance of M2 cells could be related to the immunomodulatory capabilities of SC, which have been reported to be able to regulate the M1/M2 cell balance, with activation of M2 cells.[30]

In addition, it has been reported that PP may have high cytotoxicity on human fibroblasts as compared to other commonly-used prosthetic biomaterials.[31] Furthermore, PP biomaterials may increase oxidative stress in cultured cells, which is in relation with inflammatory potential.[31] However, the use of novel SCTLAS was able to reduce this inflammatory reaction in host tissues 14 days after grafting and at day 28 with PP/PG-SCTLAS. This could be explained by the presence of SC in the SCTLAS. Although these cells still need further characterization, previous works demonstrated that stromal cells fulfill some of the ISCT minimal criteria for adult stem cells, including the capability for multipotent differentiation to osteogenic, chondrogenic, and adipogenic lineages.[32,33] These cells could release a large number of growth factors including cytokines, chemokines involved in migration, expansion, and factors involved in immunomodulation and angiogenesis,[33,34] as it has been described for mesenchymal SC.[35,36] SC Moreover, the use of natural biomaterials as fibrin and agarose as scaffold may enhance the biocompatibility and minimize the inflammatory effects of SM. In this sense, some previous studies reported the relevance of cellular tissue substitutes able to reduce the inflammatory response and to increase neovascularization and tissue regeneration as compared to acellular matrices.[37] The fact that PP-SCTLAS tissues were not different to SM at day 28 may imply that the inflammatory reaction driven by PP-SM tends to decrease after 1 month post-implant. Previous reports using other types SM immersed in novel hydrogels demonstrated that SM coating significantly reduced the number of inflammatory cells in vivo and can modulate the acute response.[37,38]

To assess the quality of the tissues regenerated in vivo and to determine if these tissues are similar to native structures, we analyzed the fibrillar and non-fibrillar components of the ECM in the newly formed tissues. Synthesis of normal ECM components is one important aspect that a stroma-like tissue should fulfill. After culturing SCTLAS in vitro for 35 days, most of the ECM components, including fibrillar and non-fibrillar molecules, were negative or slightly positive, with no differences among groups. However, most ECM components increased once SCTLAS were grafted on animals, suggesting that the in vivo environment, including a number of paracrine signals, is necessary for the full development and differentiation of the bioengineered tissues. This phenomenon has been previously found in bioengineered tissues kept in vitro, which are usually devoid of most ECM components while kept in culture.[22,39]

One of the main problems associated to the in vivo use of SM is the generation of an intraabdominal fibrotic process.[20] In this regard, non-absorbable biomaterials could induce the formation of fibrotic tissue within and around the SM, with the synthesis of large amounts of fibrillar components of the tissue ECM. This kind of reaction can lead to chronic discomfort, perforations, and bowel obstruction.[40–42] Therefore, development of novel abdominal meshes based on current regenerative medicine approaches able to decrease scar tissue deposition would have high clinical impact.[42] In our study, the analysis of collagen synthesis and accumulation demonstrated that the use of SCTLAS was significantly associated to lower collagen expression around the fibers of the PP-SCTLAS and PP/PG-SCTLAS fibers, than SM. Previous works already showed that SM, especially PP, can induce a fibrotic reaction at the grafting site, and the use of novel biological biomaterials combined with the SM such as chitosan-coating of PP may reduce the fibrotic reaction.[38] Future studies should determine the role of the cells included in the SCTLAS in the prevention of the fibrotic reaction mediated by cytokines expression.[29] Our histologic results also revealed higher presence of collagen and other ECM fibers such as elastic fibers when SM was grafted alone, although no differences were found for reticular fibers, and these dense ECM components were found encapsulating the PP-SM or PP/PG-SM filaments. The use of SCTLAS not only resulted in lower presence of collagen, but also in a more regular fibrillar pattern within the SCTLAS, resembling the native tissue. All these findings make us suggest that SCTLAS are associated to a process of tissue regeneration leading to structures that are more similar to the control abdominal wall as compared to SM. Regarding non-fibrillar ECM components, we found that the presence of glycoproteins and proteoglycans as determined by PAS and alcian blue staining, respectively, did not differ between SM and SCTLAS. This finding could imply that non-fibrillar components are more stable and do not depend on the nature of the material used, as fibrillar components did.

Interestingly, our analysis of cell migration found that SM grafting was associated to a high percentage of cells positive for cortactin. As demonstrated by several authors,[43,44] cortactin plays a complex role in cellular migration and invasion, promoting actin polymerization and cytoskeletal and membrane protein trafficking. In addition, MMP14 proteins represent essential components of the cellular machinery involved in the dissolution and remodeling of the ECM,[45] and it has been recently associated to cell extensions and podosomes,[46] and pericellular proteolysis could be mediated by MMP14. In our analysis, SM samples showed a high number of cells expressing cortactin and high MMP14 expression, suggesting that cells tend to migrate to the grafting site and to remodel the native ECM, thus contributing to the inflammatory process and fibrotic

reaction described above. In contrast, SCTLAS grafting resulted in less cortactin and MMP14 expression, with levels resembling the native control more than SM. In consequence, the use of SCTLAS could contribute to a more physiological cell response, with less cell migration and ECM remodeling, and this could be related to.[47]

In summary, our results suggest that the use of novel SCTLAS based on SC and tissue engineering methods could contribute to prevent the tissue inflammation and excessive fibrotic reaction phenomena found in control SM while maintaining the biomechanical properties of SM. Although these results are promising and point to the possible clinical translational potential of these SCTLAS, advanced therapies clinical trials should be carried out in this field to determine if these novel products are clinically useful.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no commercial or financial conflict of interest.

Keywords

abdominal wall repair, artificial stroma, stromal cells, surgical mesh, tissue engineering

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