

Sutures enriched with adipose-derived stem cells decrease the local acute inflammation after tracheal anastomosis in a murine model[†]

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Abstract

OBJECTIVES: Local inflammatory reaction and tension are the main causes of postoperative complications after tracheal surgery. Adipose-derived stem cells (ASCs) are known to have immunomodulatory activity. The exact mechanism of this activity is not known, although it is possible that they modulate the function of different cells involved in the immune response. Little is known of their impact on acute inflammation, especially in the problematic tracheal area. We aimed to study the effect of ASCs applied locally in an animal model of tracheal resection and anastomosis.

METHODS: ASCs from the subcutaneous fat of BDIX rats were infected for expression of the enhanced green fluorescent protein (eGFP) and were cultured with Polyglactin 910 sutures to obtain biosutures (ASC-coated sutures). After tracheal resection, 90 BDIX rats (syngeneic, autologous model) underwent anastomosis with biosutures (1.5×10^6 cells/biosuture [Group 1] or 0.5×10^6 cells/biosuture [Group 2]) or conventional sutures (Group 3). The animals were killed after 1, 4, 10, 30 or 60 days and histological and immunofluorescence studies were performed on the anastomotic areas. Inflammatory cell densities were graded semiquantitatively by the pathologist in a blinded fashion.

RESULTS: In the early period (1 and 4 days), the biosuture groups presented an atypical pattern of acute inflammation, characterized by the almost complete absence of neutrophils, and the presence of abundant lymphocytes and plasma cells, compared with the control group ($P < 0.05$). Moreover, abundant macrophages/monocytes were immunolocalized around blood vessels near the biosutures and between biosuture threads 1 day after anastomosis, whereas the presence of macrophages/monocytes in animals treated with conventional sutures was discrete ($P < 0.05$). No differences were observed in the later period. No side effects in the biosuture groups were found.

CONCLUSIONS: Biosutures are a comfortable way of stem cell delivery to the surgical field without modification of the operative protocol. ASCs suppress the local acute inflammatory reaction (increased macrophage migration and decreased neutrophil infiltration) in the tracheal anastomosis and cause an early switch from acute to chronic inflammation.

Keywords: Mesenchymal stem cells • Adipose-derived stem cells • Tracheal surgery • Tracheal anastomosis • Biosutures

INTRODUCTION

Mesenchymal stem cell (MSC) research has developed rapidly during the last decade and the promising results obtained from *in vitro* and *in vivo* studies have generated growing optimism. These cells were distinct from the haematopoietic stem cells and could be identified by their capacity to adhere to plastic, to

generate colony-forming unit fibroblasts in culture, and their potential to differentiate into several lineages such as bone and cartilage cells and adipocytes [1]. It is also assumed that MSCs can undergo self-renewal, in particular since they can be maintained in culture for prolonged periods of time. Although bone marrow is the most often used source, MSCs with similar biological properties have also been isolated from other tissues including adipose tissue, skeletal muscle and cord blood [2–5]. Of special interest is adipose tissue since it represents an abundant and accessible source of MSCs. These cells are denominated adipose-derived stem cells (ASCs) and have been widely studied since they were first described in 2001 [5]. In recent

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years, substantial knowledge of ASCs and MSCs interaction with the immune system has been acquired. The mechanisms underlying the immunosuppressive effects of ASCs have not been clearly defined, but it seems that ASC modulates the function of different cells involved in the immune response [6, 7].

Recent studies address the potential benefit of application of MSCs in systemic acute inflammatory response in septic shock [8–11]. However, little is known about the impact these cells could have on the local acute inflammation. In this first line of defence, the immune response is nonspecific and characterized by changes in microcirculation: exudation of fluid and migration of leukocytes from blood vessels to the area of injury. Neutrophils are the main effectors and the most predominant cell type during the first 24 h. Macrophages migrate to the damaged tissues after the arrival of neutrophils and their number slowly increases facilitating the elimination of dead material after 2–3 days of neutrophil activity. Other cell types (lymphocytes, plasma cells, etc.) are also involved in the initial inflammatory reaction and, together with the increasing number of macrophages, represent the most prominent cells in chronic inflammation.

Acute inflammatory reaction is a double-edged sword. It plays a key role in the initial host defence response, particularly against many infections, but its aim is imprecise and, as a consequence, when it is drawn into battle, it can cause collateral damage in tissues and impair healing.

Different methods have been proposed for enhancing anastomotic healing after tracheal resection and anastomosis, such as sealants, different types of sutures, tension-relieving techniques and tissue transpositions. Nevertheless, anastomotic complications (mainly stenosis and dehiscence) occur in around 9% of the patients and remain a major concern [12]. Local inflammatory reaction is found to be among the most important causes of postoperative complications [13, 14]. Corticoids have been used in some studies in an attempt to decrease this reaction, but they might also impair healing [15] and so are used with serious consideration of possible complications. Tension on the anastomotic area and poor vascular supply are the other main causes of postoperative complications.

We hypothesized that some of the above-mentioned properties of ASCs could be involved in the earlier stages of the immune response and modulate the local acute postoperative inflammation. For that purpose, we used an animal model of tracheal resection and anastomosis, and compared the application of biosutures (sutures enriched with ASCs) with conventional sutures.

METHODS

Isolation and culture of adipose-derived stem cells

ASCs were obtained from the subcutaneous fat tissue of BDIX rats according to a previously described protocol in humans with minor modifications [5]. Subcutaneous fat tissue from two BDIX male rats was ground into small pieces and digested with type I collagenase (0.075%; Gibco BRL). The digested tissue was centrifuged at 250 g for 10 min and the pellet was resuspended in 0.16 M NH₄Cl for erythrocyte lysis. After filtering through a 70- μ m nylon mesh, the cells were plated in culture dishes and

cultured at 37°C in a humid atmosphere with 5% carbon dioxide in the Dulbecco's modified Eagle's medium (Gibco BRL) containing 10% foetal bovine serum (Gibco BRL) and 1% penicillin–streptomycin (Gibco BRL). The medium was changed to remove nonadherent cells 24 h after seeding, and every 4 days thereafter. For subculturing, cells were detached with 0.05% (v/v) trypsin in phosphate-buffered saline when 70–80% confluence was reached. In the third subculture, when 60–70% confluence was reached, ASCs were infected with lentivirus (CNIC, Madrid, Spain) to transduce the enhanced Green Fluorescent Protein (eGFP). Cultured cells were analysed up to passage 15 to ensure >95% of eGFP expression by flow cytometry. In this study, ASCs were applied after 5–6 passages.

Characterization of adipose-derived stem cells

Cell cultures were analysed by four-colour flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San José, CA, USA) after staining with fluorochrome-conjugated monoclonal antibodies. The following conjugated antibodies were used at saturating quantities: Alexa Fluor 647 conjugated CD90, CD29, CD45 and CD11b (Serotec, Spain). Briefly, 2×10^5 nucleated cells were incubated for 20 min at room temperature with the above-mentioned antibodies. Data acquisition and analysis were performed using the CELLQuest PRO software program (BD Biosciences).

Biosuture preparation

Biosutures were obtained by culturing 6/0 Polyglactin 910 suture (Vicryl™; Ethicon, Edinburgh, UK) with ASCs–eGFP on ultra-low attachment plates (Costar; Corning, New York, USA) according to a previously published protocol with minor changes [16]. Two different quantities of ASCs–eGFP were used— 1.5×10^6 cells/suture and 0.5×10^6 cells/suture. The cultures were maintained for 72 h in standard conditions, then washed with saline and used for tracheal anastomosis. At these conditions, cell viability was evaluated by trypan blue.

Cell adherence to the suture and surgical needle was confirmed by fluorescence microscopy and electron microscopy. In a preliminary study, we examined the biosutures at different times—before application and after one, two or more stitches done with the same suture and passing them through different tissues. We found that cells density significantly decreased after two stitches in muscle tissue, and thus we decided to use each suture for only two stitches.

Surgery

The present study was performed in accordance with the European Union guidelines for reducing pain and discomfort to experimental animals. The study protocol was approved by the Ethical Committee for Animal Welfare (Comité Ético de Bienestar Animal, Protocol No. 05-06) of our hospital.

Ninety adult male BDIX (syngenic) rats (mean weight 311 g) were used in order to mimic an autologous stem cell application. Resection of three tracheal rings and anastomosis was done after intraperitoneal anaesthesia using a mixture of ketamine (100 mg/kg), atropine (0.4 mg/kg) and diazepam (8 mg/kg). Tracheal

anastomosis was performed with six interrupted stitches (two stitches with each suture, three sutures/anastomosis). Animals were distributed in the following experimental groups: Group 1, biosuture with 1.5×10^6 cells/suture ($n = 30$); Group 2, biosuture with 0.5×10^6 cells/suture ($n = 30$) and Group 3, suture without cells ($n = 30$). The animals were reanaesthetized and killed by an intraperitoneal overdose of pentobarbital at Days 1, 4, 10, 30 or 60 and the tracheas were extracted for further histological and immunofluorescence studies.

Histopathology

The tracheas were fixed in 4% formaldehyde and embedded in paraffin. Slices 6- μ m thick were stained with haematoxylin and eosin and examined under a Leica DM LS2 microscope by a pathologist who was blinded to the study group. The following features were evaluated semi-quantitatively to define the inflammatory pattern: presence of neutrophils, lymphocytes and plasma cells, macrophages, activated fibroblasts, fibrin deposits and tissue oedema. Micrographs were taken with a digital camera (Leica EC3). Representation of each cell type was graded by the pathologist on a blinded fashion as follows: 0, absence; 1, low density; 2, medium density; 3, high density. The inflammatory pattern was informed for each specimen according to the cellularity (acute, early or advanced chronic inflammation). In cases of deviation from the normal pattern, a more detailed report was provided.

Immunofluorescence

Sections 6- μ m thick were deparaffinized, rehydrated and heat pretreated with citrate buffer (pH 6) for immunofluorescence with antiGFP antibody (SC-8334; Santa Cruz Biotechnology, CA, USA) or trypsin pretreated for double immunofluorescence with antiGFP antibody and antimacrophage/monocyte (CD68) antibody (MAB-1435; Millipore, CA, USA). Thereafter, the sections were incubated with blocking buffer for 1 h and primary antibodies diluted 1:100 were added. The mixture was then incubated overnight. This step was followed by washing and incubation for 1 h with the secondary antibody, anti-rabbit Alexa Fluor 488 (Molecular Probes; Eugene, OR, USA) diluted 1:200 for immunofluorescence with antiGFP or in combination with anti-mouse Alexa Fluor 544 (Molecular Probes; Eugene) diluted 1:200 for double immunofluorescence. The sections were mounted with antifade reagent with 4',6-diamidino-2-phenylindole (DAPI) (Prolong Gold; Molecular Probes, Eugene) and viewed under a fluorescent microscope (Leica DMI6000B). Primary antibody omitted sections were used as the negative controls.

Statistical analysis

The Mann-Whitney *U*-test was used to compare the cell confluence of the inflammatory cells among the studied groups. $P < 0.05$ was considered statistically significant.

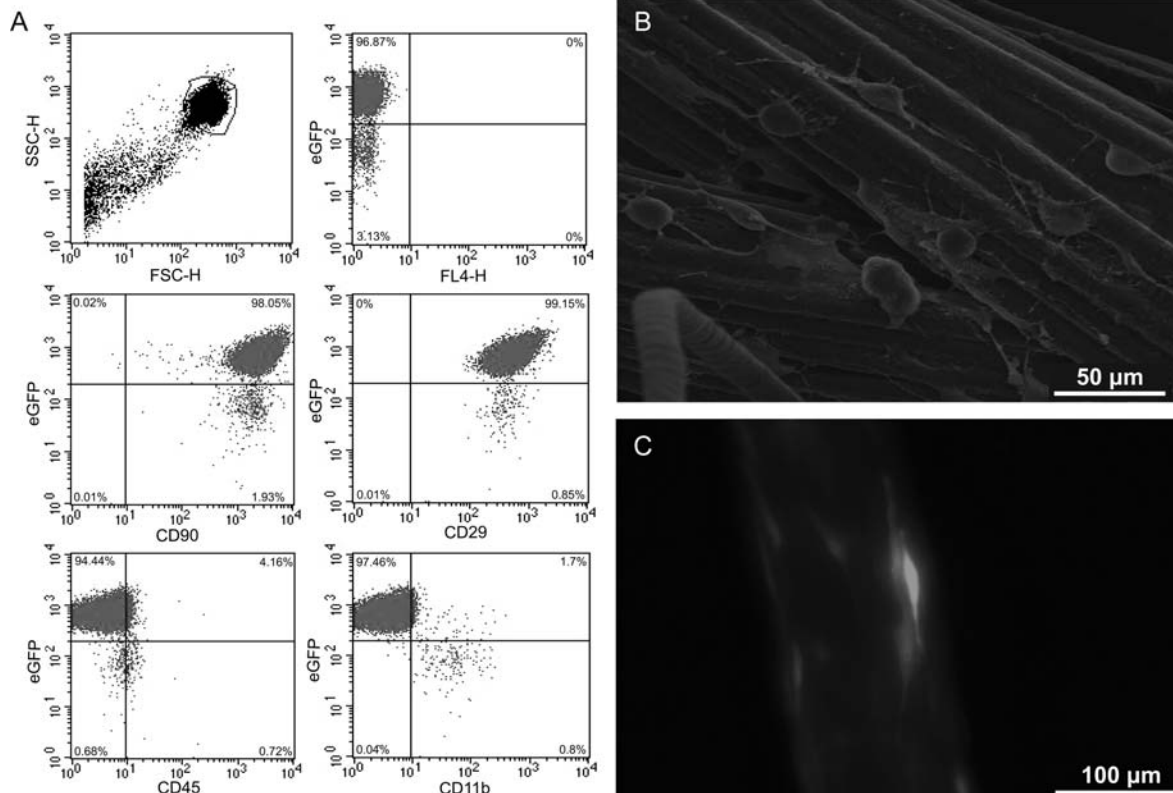


Figure 1: The characterization of ASCs and biosuture preparation. (A) Representative flow cytometry dot plots showing the high expression of eGFP (>95%), CD90 (>95%) and CD29 (>95%) and lack of surface markers CD45 (<5%) and CD11b (<5%), confirming the mesenchymal phenotype of cultured cells. (B) Electron microscopy of the biosuture showing ASCs between the suture threads. (C) Fluorescence microscopy of a biosuture cultured for 24 h with 0.5×10^6 ASCs showing eGFP-positive cells attached to the suture.

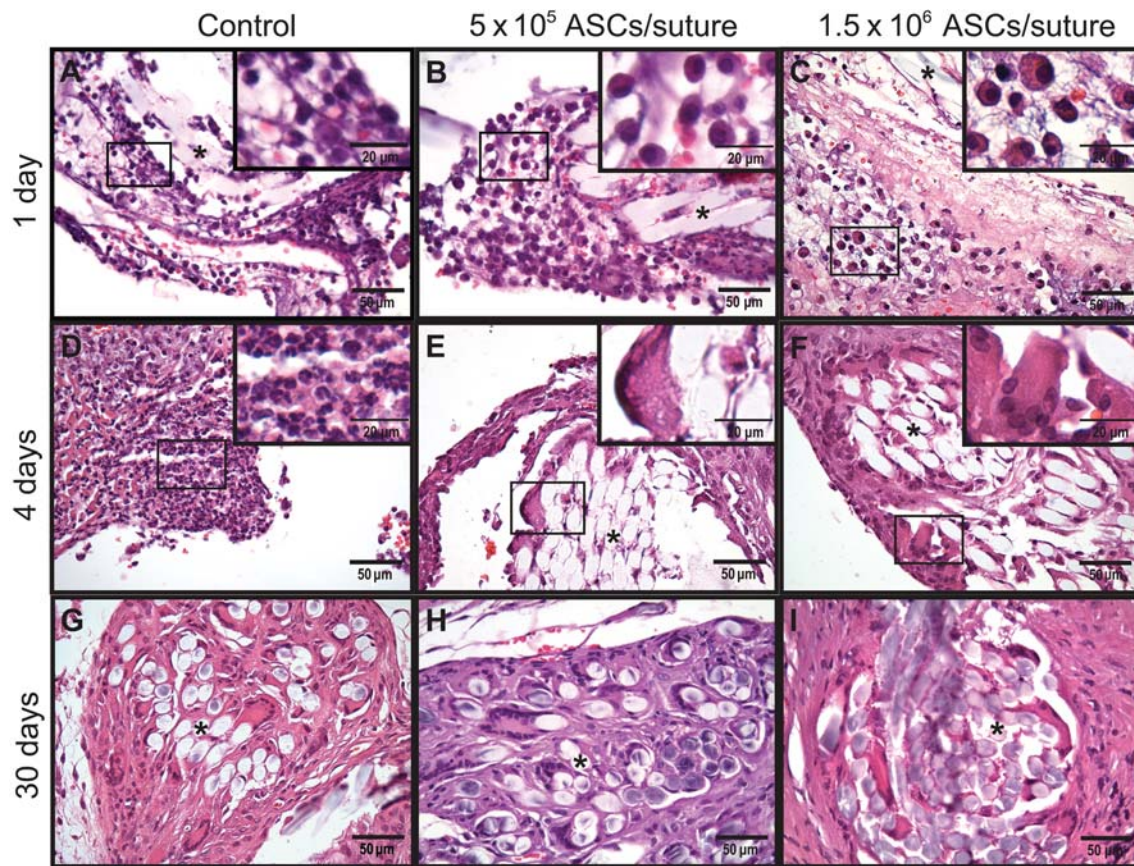


Figure 2: Haematoxylin and eosin stained slices from the anastomotic area of the tracheas at Days 1, 4 and 30 after anastomosis. Asterisk: surgical suture. (A and D) A typical acute inflammatory pattern with abundant neutrophils was found in anastomoses of the control groups (without ASCs) at Days 1 and 4. The neutrophils were observed between the fibres of the suture (inset of A) and infiltrating the surrounding tissues (inset of D). (B, C, E and F) An atypical acute inflammatory pattern was found in the anastomoses of the biosuture groups at Days 1 and 4, characterized by the almost complete absence of neutrophils and the presence of plasma cells attached to the suture (ASCs) (insets of B and C). In most of the biosuture group at Day 4, a complete absence of neutrophils and presence of multinucleated cells engulfing the suture fibres (early chronic granulomatous inflammatory pattern) were observed (insets of E and F). This pattern was also found in all the anastomoses of the biosuture and control groups at Day 10. (G, H and I) An intermediate chronic granulomatous inflammatory pattern was found in anastomoses at Day 30 with no differences between biosuture and control groups. There were no histological differences between the biosuture groups.

Table 1: Cellularity comparison between the biosuture groups

Time	Group ($\times 10^6$ cells/suture)	NE	P	LY + PC	P	MF	P	MNC	P
1 day	1.5	0.8 (0.45)	0.317*	3.0 (0.00)	1000*	1.6 (0.55)	0.513*	0 (0.00)	1000*
	0.5	1.0 (0.00)		3.0 (0.00)		1.8 (0.45)		0 (0.00)	
4 days	1.5	0.2 (0.45)	0.221*	1.8 (0.84)	1000*	2.6 (0.55)	0.549*	1.6 (0.89)	0.221*
	0.5	0.6 (0.55)		1.8 (0.84)		2.4 (0.55)		0.8 (1.10)	
10 days	1.5	0 (0.00)	0.317*	0.8 (0.45)	1000*	3.0 (0.00)	0.317*	2.0 (0.00)	0.317*
	0.5	0.2 (0.45)		0.8 (0.45)		2.8 (0.45)		2.2 (0.45)	
30-60 days	1.5	0 (0.00)	1000*	0 (0.00)	1000*	0.5 (0.53)	0.374*	3.0 (0.00)	1000*
	0.5	0 (0.00)		0 (0.00)		0.3 (0.48)		3.0 (0.00)	

Data is represented as mean (standard deviation) of the cellularity scores (0, absence; 1, low density; 2, medium density; 3, high density) graded by the pathologist on a blinded fashion. NE: neutrophils; LY + PC: lymphocytes and plasmatic cells; MF: macrophages; MNC: multinuclear cells; *: nonsignificant.

RESULTS

Characterization of adipose-derived stem cells and biosuture preparation

Flow cytometry analysis confirmed eGFP expression in the cell culture (>95%). The mesenchymal phenotype was confirmed by

the high expression (>95%) of CD90 and CD29 and the absence (<5%) of CD45 and CD11b surface markers (Fig. 1A). The biosuture was successfully prepared as shown in Fig. 1B and C in which cells between the suture threads can be observed. Cell viability (> 98%) was confirmed by trypan blue. More than 80% of the cultured cells were attached to the suture. At the time of application, biosutures were undistinguishable and their handling

Table 2: Cellularity comparison among groups

Time	Group	NE	P	LY + PC	P	MF	P	MNC	P
1 day	B	0.9 (0.32)	0.001	3.0 (0.00)	0.001	1.7 (0.48)	0.013	0 (0.00)	NS
	C	3.0 (0.00)		1.0 (0.00)		1.0 (0)		0 (0.00)	
4 days	B	0.4 (0.52)	0.001	1.8 (0.798)	NS	2.5 (0.53)	0.001	1.2 (1.03)	0.031
	C	2.8 (0.45)		1.2 (0.45)		1 (0)		1 (0)	
10 days	B	0.1 (0.32)	NS	0.8 (0.42)	NS	2.9 (0.32)	NS	2.1 (0.32)	NS
	C	0.4 (0.55)		0.8 (0.42)		2.4 (0.89)		2.0 (0.71)	
30–60 days	B	0 (0.00)	NS	0 (0.00)	NS	0.4 (0.50)	NS	3.0 (0.00)	NS
	C	0 (0.00)		0 (0.00)		0.3 (0.48)		3.0 (0.00)	

Data is represented as mean (standard deviation) of the cellularity scores (0, absence; 1, low density; 2, medium density; 3, high density) graded by the pathologist on a blinded fashion. B: biosutures; C: controls; NE: neutrophils; LY + PC: lymphocytes and plasmatic cells; MF: macrophages; MNC: multinuclear cells; NS: nonsignificant.

was equivalent to the conventional sutures. Taking into account that more than 80% of the cells adhere to the suture, we obtained sutures that carried at least than 1.2×10^6 cells (when cultured with 1.5×10^6 cells) and 0.4×10^6 cells (when cultured with 0.5×10^6 cells). As we used 30 cm sutures, each suture carried at least 4000 cells/mm and 1300 cells/mm, respectively, although the cells were not homogeneously distributed along the suture and the needle carried some cells too.

Histopathological findings

Typical acute inflammation was observed in the control group anastomoses at 1 and 4 days (Fig. 2A and D), characterized by extensive fibrin deposits around the tracheal wound and the surgical suture mixed with abundant neutrophils, some lymphocytes, plasmatic cells and macrophages. Some neutrophils were identified dissecting the fibres of the surgical suture. Blood capillaries and tissue oedema with activated fibroblasts in the neighbouring areas were also prominent. However, at 1 and 4 days, an atypical acute inflammatory pattern was observed in the biosuture groups (1.5×10^6 cells/suture and 0.5×10^6 cells/suture; Fig. 2B, C, E and F), characterized by numerous plasmatic cells dissecting the fibres of the surgical suture and presence of abundant macrophages and scant neutrophils in the surrounding areas. Fibrin deposits, blood capillaries, tissue oedema and fibroblasts were less prominent.

Half of the anastomoses of the biosuture groups presented an early chronic granulomatous inflammatory reaction at 4 days; this reaction was evident in all groups at 10 days (Fig. 2E and F) and was characterized by abundant macrophages and some giant multinucleated cells around and between the fibres of the surgical suture trying to engulf it, together with numerous activated fibroblasts. There were also lymphocytes, plasma cells and scant neutrophils in the neighbouring areas, as well as some eosinophils.

At 30 and 60 days after the procedure, all the groups presented an advanced chronic granulomatous inflammation pattern with the whole surgical suture completely engulfed by a variable amount of collagen and fully developed granulomas (abundant giant multinucleated cells phagocytosing the isolated fibres of the surgical suture; Fig. 2G, H and I). There was no evidence of neoplastic processes at any stage.

The Mann-Whitney *U*-test was initially performed to compare the cellularity of the two experimental groups (1.5×10^6 cells/suture and 0.5×10^6 cells/suture) at the studied time points. As there were no statistically significant differences between them (see Table 1), both experimental groups were studied together as a 'biosuture group' for further statistical analysis.

Cell confluence of the inflammatory cells for each group is shown in Table 2. When biosutures were applied, an increased macrophage migration ($P = 0.013$ and 0.001) and decreased neutrophil infiltration ($P = 0.001$ and 0.001) was found at Days 1 and 4, respectively. Multinuclear cells were found earlier (at 4 days) in the biosuture groups while they were absent in the controls ($P = 0.031$).

Immunofluorescence

ASCs-eGFPs were still present in the sections obtained from the anastomotic areas from both biosuture groups even at 10 days after the procedure. Most ASCs-eGFP remained attached to the sutures, within the foreign body granuloma adjacent to and around the suture (Fig. 3A and B). Some ASCs-eGFPs could, however, be found within the perichondrium at Day 10 after the procedure (Fig. 3C). No cells were found within the tracheal epithelium. Stem cells were not detected at posterior follow-up sections.

Macrophages/monocytes were immunodetected in the tracheal anastomosis area of all the groups. Nevertheless, some differences were appreciated at Day 1 after the anastomosis in the biosuture groups. In the groups with normal sutures, there was only a discrete presence of macrophages/monocytes around the suture fibres (Fig. 3D). In contrast, in the biosuture groups, there was an abundance of macrophages/monocytes widely distributed near and between the biosutures where ASCs-eGFP were detected (Fig. 3E). Moreover, high amounts of macrophages/monocytes were immunolocated around blood vessels, situated in the proximity of the biosutures (Fig. 3F). No differences were found at later periods.

DISCUSSION

We report, for the first time, that ASCs applied *in situ* can modify the local acute inflammatory pattern in an animal model of

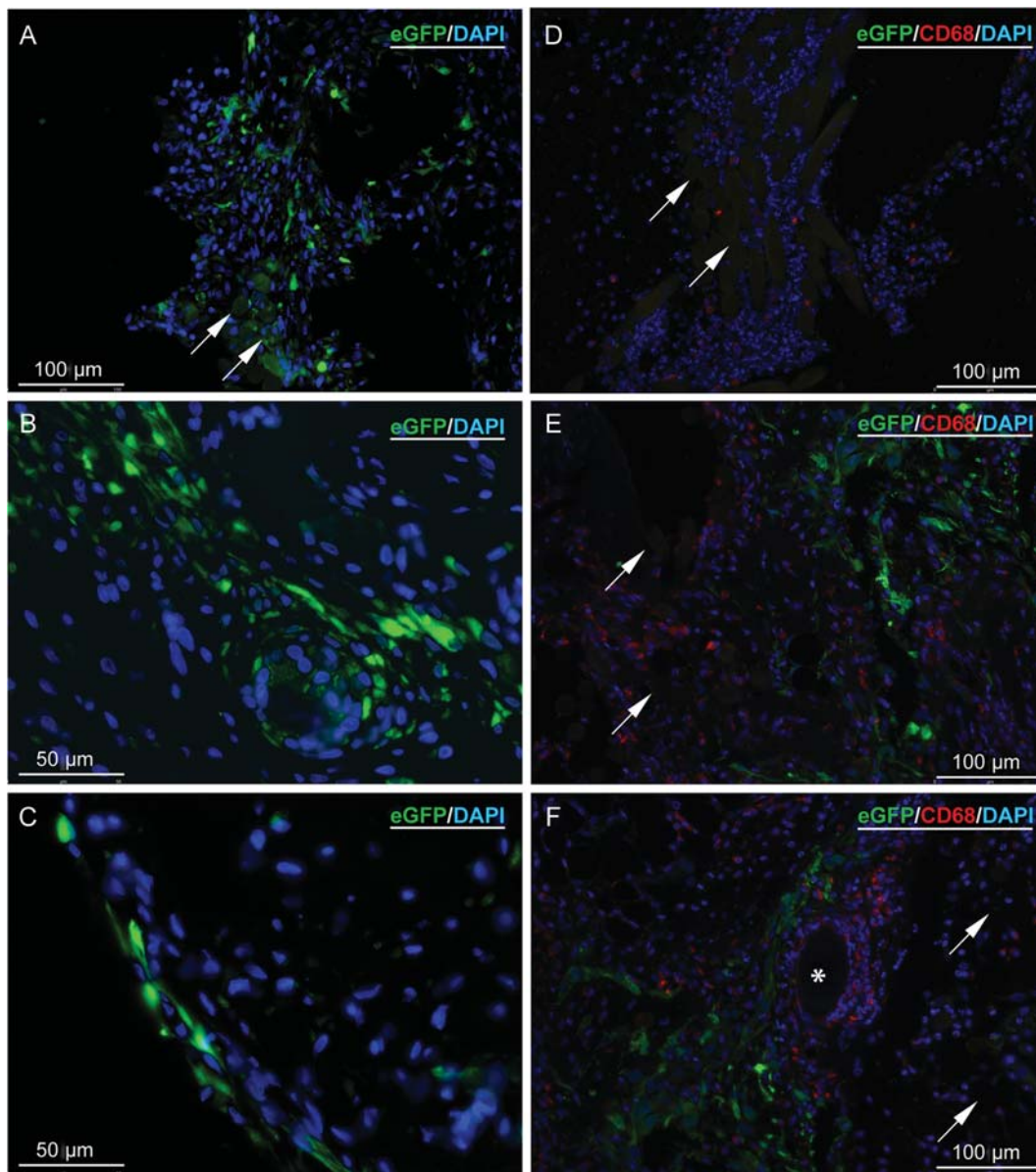


Figure 3: Immunolocalization of ASCs and macrophage/monocyte (CD68) at the surgical site after tracheal resection and anastomosis with biosutures. Cell nuclei were stained with DAPI (blue). (A) eGFP-positive cells (green) around the suture (white arrows) at 1 day after the anastomosis. (B) Foreign body granuloma of the suture with eGFP-positive cells (green) present within 4 days of anastomosis. (C) eGFP-positive cells (green) located at the level of the perichondrium 10 days after anastomosis. (D) There was a discrete presence of macrophages/monocytes (CD68) (red) around the suture fibres (white arrows) on the first day after the anastomosis with normal sutures (control group). (E) Abundance of macrophages/monocytes (CD68) (red) widely distributed in nearby tissue and between the biosutures (white arrows) where eGFP-positive cells (green) were detected 1 day after anastomosis. (F) High amounts of macrophages/monocytes (CD68) (red) were immunolocalized around blood vessels (asterisk), situated in the proximity of the biosutures (white arrows) 1 day after anastomosis.

tracheal resection and anastomosis. Abundant macrophage infiltration and only scant neutrophils were observed in the anastomotic site of the biosuture groups, at the earliest phases of the inflammatory reaction (1 and 4 days), while the control groups featured abundant neutrophils ($P < 0.05$) and only scant macrophages ($P < 0.05$). Although ASCs were found within the anastomotic area 1 month after surgery, no differences of the inflammatory pattern were detected at more advanced phases of the inflammatory reaction (at 10, 30 and 60 days; Table 2).

The immunomodulatory activity of ASCs is well studied and has been exploited clinically. In animal models and clinical settings, this activity is thought to be largely based on inhibition of the T-cell and B-cell proliferation and dendritic cell maturation

observed *in vitro* and *in vivo* [17] and secretion of a large number of cytokines and growth factors (epidermal growth factor, keratinocyte growth factor, insulin-like growth factor-1, vascular endothelial growth factor-A, platelet-derived growth factor-BB, erythropoietin and thrombopoietin) [18]. Some of these are found to have a beneficial effect in tracheal surgery (vascular endothelial growth factor) [19]. However, little is known about their role in acute inflammation. A study by Nemeth *et al.* [10] published in 2009 reported that MSCs were able to attenuate sepsis by prostaglandin E2-dependent reprogramming of macrophages to increase production of IL-10, an inflammatory cytokine. Monocyte and macrophage-derived IL-10 seems to prevent neutrophils from migrating into tissues and causing

oxidative damage, thus mitigating organ damage. This study showed that the beneficial effect is likely to be due to increased release of prostaglandin E2 from MSCs, which acts on EP2 and EP4 receptors of macrophages to stimulate production and release of IL-10. Recent *in vitro* studies hypothesize that macrophages are a major type of effector cell through which MSCs might exert their therapeutic effects. It was found that MSCs induce macrophages to switch to an anti-inflammatory profile, characterized by increased phagocytic activity, high expression of IL-10 and IL-6 and low expression of IL-12 and tumour necrosis factor-alpha [20, 21]. Our results, in line with these studies, show that when ASCs are applied topically (biosutures) to the tracheal anastomosis, neutrophils are almost absent and abundant macrophages appear earlier in the damaged tissues in the early phases of acute inflammation. Comparing changes in the inflammatory reaction between the groups with and without biosuture, the biosuture groups showed an earlier 'switch' from an acute to a chronic inflammatory pattern (on the first instead of fourth or fifth day) that could be beneficial for healing (Fig. 4). Anastomotic inflammation is thought to be one of the most important causes of postoperative complications after tracheal resection [14]. It therefore follows that the inhibition of acute inflammation could improve the outcomes. The neutrophils at the surgical site are responsible for removing necrotic tissues and bacteria and produce a wide variety of factors. However, this reaction is unspecific, and neutrophils could cause damage to the normal surrounding tissues [22, 23]. Surgeons are well aware that inflamed tissues are more fragile and healing is slower, as seen in some paradigmatic inflammatory diseases like inflammatory bowel disease. Tension on the anastomotic line (generally modifiable by surgical techniques) and poor vascular supply are the other main causes of anastomotic complications. Angiogenic properties of MSCs could potentially play an interesting role in improving the vascular supply, but there are no published studies that demonstrate this mechanism on the anastomotic area.

Based on our histopathological findings, we think that ASC application could improve the tracheal healing process during the first postoperative days, when the most clinically relevant problems (dehiscence, leaks, etc.) tend to occur. In our study, we achieved the attenuation of the early acute inflammatory reaction with no effect on the more advanced stages of inflammation and healing. This study is only an example of the potential

spectrum of applications that could be studied, not only in the field of thoracic surgery. Our pioneer idea was to target the procedures where the conventional sutures play a critical role, and the consequences of suture failure could be devastating. We think that those applications could be all kind of anastomoses. Our team have previously published two papers on the use of biosutures in colonic anastomosis, where the biosuture groups had similar strength patterns with less perianastomotic adhesions [16], and in case of critical anastomosis (without adhesions, after icodextrin 4% application), they provided an increased anastomotic resistance [24]. Tracheal anastomoses, from our point of view, are another playground where biosutures could be helpful. Other possible applications in the thoracic surgery field could be tracheoesophageal fistula, bronchial stump closure and other reconstructive techniques.

Previous studies concluded that wound healing might be attributed to angiogenesis and differentiation of the applied and probably also resident MSCs [25]. In our experiments, the immunofluorescence studies identified ASCs within the perichondrium (10 days) and close to the anastomotic edges and surgical suture (Fig. 3). Although MSCs are known to be capable of epithelial differentiation, we did not observe ASCs at the level of tracheal epithelium.

We have studied two kinds of biosutures with different cell count as neither the experimental models nor the clinical practice of different diseases have provided an approximation to the best dose or at least the minimal dose 'clinically active'. All the work in this field is on going. We thought that the higher cell count could produce more 'loaded' sutures and improve the results but our results did not support that theory (Table 1). Even more, we found that the higher the cell density was—the higher the possibility for the cells to form clusters instead of adhering to the suture.

In conclusion, ASCs seem to decrease the acute inflammatory reaction, as indicated by a decreased neutrophil presence and increased macrophage infiltration in the early stages after tracheal resection and anastomosis. Using a biosuture, a topical application of these cells is possible while ensuring that the surgical protocol remains unchanged. These sutures can be used in a similar fashion (in strength, usability, flexibility, etc.) to conventional sutures. The modulation of acute inflammatory reaction promoted by ASCs, preventing excessive tissue injury, opens new perspectives for improving not only wound healing but also a number of conditions where the acute inflammation is the main pathogenic pathway. Further studies are needed to describe in detail the molecular mechanisms of these effects.

No adverse reactions or neoplastic processes related to the implanted ASCs were observed during this study.

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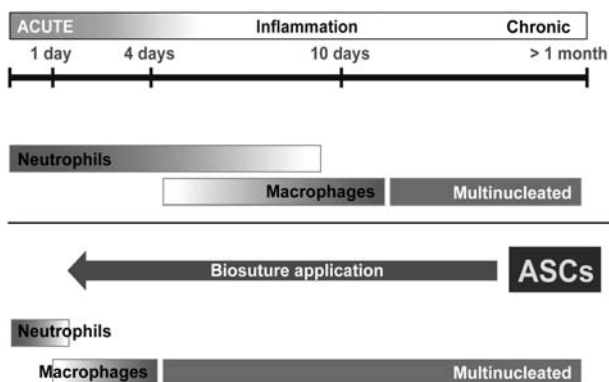


Figure 4: A schematic view of the inflammatory progression and switch from acute to chronic pattern at standard conditions (upper part of the figure) and after ASC (biosutures) application (lower part of the figure).

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Conflict of interest: Damián García-Olmo is a co-holder of patent rights related to biomaterial for suturing (P200402083 in Spain, 04380271.9 in Europe and 101573.55823US in the USA). There are no other ethical problems or conflicts of interest.

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