Published in final edited form as:

Exp Eye Res. 2010 July ; 91(1): 92–96. doi:10.1016/j.exer.2010.04.007.

Corneal Myofibroblast Generation From Bone Marrow-derived Cells

Flavia L. Barbosa¹, Shyam S. Chaurasia^{1,2}, Alicia Cutler¹, Kewal Asosingh³, Harmet Kaur¹, Fabricio W. de Medeiros^{1,4}, Vandana Agrawal¹, and Steven E. Wilson^{1,*}

¹ Cole Eye Institute, The Cleveland Clinic, Cleveland, OH

² Singapore Eye Research Institute, Singapore

³ Department of Pathobiology, Lerner Research Institute, The Cleveland Clinic, Cleveland, OH

⁴ Department of Ophthalmology, University of Sao Paulo, Sao Paulo, Brazil

Abstract

The purpose of this study was to determine whether bone marrow-derived cells can differentiate into myofibroblasts, as defined by alpha smooth muscle actin (SMA) expression, that arise in the corneal stroma after irregular phototherapeutic keratectomy and whose presence within the cornea is associated with corneal stromal haze. C57BL/6J-GFP chimeric mice were generated through bone marrow transplantation from donor mice that expressed enhanced green fluorescent protein (GFP) in a high proportion of their bone marrow-derived cells. Twenty-four GFP chimeric mice underwent haze-generating corneal epithelial scrape followed by irregular phototherapeutic keratectomy (PTK) with an excimer laser in one eye. Mice were euthanized at 2 weeks or 4 weeks after PTK and the treated and control contralateral eyes were removed and cryo-preserved for sectioning for immunocytochemistry. Double immunocytochemistry for GFP and myofibroblast marker alpha smooth muscle actin (SMA) were performed and the number of SMA+GFP+, SMA+GFP–, SMA–GFP+ and SMA–GFP– cells, as well as the number of DAPI+ cell nuclei, per 400X field of stroma was determined in the central, mid-peripheral and peri-limbal cornea. In this mouse model, there were no SMA+ cells and only a few GFP+ cells detected in unwounded control corneas. No SMA+ cells were detected in the stroma at two weeks after irregular PTK, even though there were numerous GFP+ cells present. At 4 weeks after irregular PTK, all corneas developed mild to moderately severe corneal haze. In each of the three regions of the corneas examined, there were on average more than 9X more SMA+GFP+ than SMA+GFP– myofibroblasts. This difference was significant ($p < 0.01$). There were significantly more ($p < 0.01$) SMA–GFP+ cells, which likely include inflammatory cells, than SMA+GFP+ or SMA+GFP– cells, although SMA–GFP– cells represent the largest population of cells in the corneas. In this mouse model, the majority of myofibroblasts developed from bone marrow-derived cells. It is possible that all myofibroblasts in these animals developed from bone marrow-derived cells since mouse chimeras produced using this method had only 60% to 95% of bone marrow-derived cells that were GFP+ and it is not possible to achieve 100% chimerization. This model, therefore, cannot exclude the possibility of myofibroblasts also developed from keratocytes and/or corneal fibroblasts.

* Address for correspondence and reprint request to: Dr. Steven E. Wilson, M.D., Professor of Ophthalmology, Director of Corneal Research, Cole Eye Institute, Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH 44195. wilsons4@ccf.org, Telephone: 216-444-5887, Fax: 216-445-8475.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Corneal myofibroblast generation is associated with injury to the cornea (Jester, Petroll, and Cavanagh, 1999; Mohan, et al., 2003). These cells are integral to wound contraction and regression that occur following incisional procedures such as radial keratotomy (Garana, et al., 1992) and the development of “haze” or stromal opacity following excimer laser surface ablation procedures such as photorefractive keratectomy (PRK) or phototherapeutic keratectomy (Mohan, et al., 2003; Netto, et al., 2006).

Many studies have demonstrated that myofibroblasts can develop from corneal fibroblasts *in vitro* when the cells are exposed to transforming growth factor beta (Masur, et al., 1996; Jester, et al., 1999; Jester, et al., 2002). These studies have naturally underlain the widely held dogma that myofibroblasts in the cornea are solely derived from keratocytes and their progeny cells. Recently, however, studies in many other tissues, including lung, skin and liver, have demonstrated that myofibroblasts can be derived from bone marrow-derived cells (Direkze, et al., 2003; Hashimoto, et al., 2004, Ogawa, et al., 2006). In the present study, chimeric mice expressing enhanced green fluorescent protein (EGFP) in bone marrow-derived cells are used to conclusively demonstrate that corneal myofibroblasts can originate from bone marrow-derived precursor cells.

2. Materials and methods

2.1. Animals and surgery

All animals were treated in accordance with the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The Animal Control Committee at the Cleveland Clinic approved the animal studies included in this work. . Anesthesia was obtained with an intraperitoneal injection of 130 µg ketamine and 8.8 µg xylazine per gram of body weight and 1 drop of 1% proparacaine HCl (Alcon, Ft. Worth, TX, USA) applied topically to the eye when phototherapeutic keratectomy was performed. Euthanasia was performed with an inhaled overdose of 5% isoflurane gas.

The EGFP chimera mice were generated in our laboratory using an adaptation of the method of Van Parijs et. al. (1999). In brief, C57BL/6J (Catalog 000664, The Jackson Laboratory, Bar Harbor, Maine) recipient mice received a total of 800 rads of whole body radiation in two doses (400 rads and 400 rads) 4 hours apart using a 137Cs Mark 1 irradiator (J.L. Sheperd & Associates, Glendale, CA). Recipient mice received gentamycin 5 mg/kg IP 2 days prior and 7 days after irradiation. The tibia and femur of C57BL/6-TgN(ACTbEGFP)10sb mice (Catalog 003291, The Jackson Laboratory) were isolated after euthanasia. This EGFP-expressing transgenic mouse line expresses an enhanced green fluorescent protein cDNA under the control of a chicken beta-actin promoter and cytomegalovirus enhancer in all cells except erythrocytes (Okabe, et al., 1997). The end of the bones were cut with Wescott scissors and the bone marrow was flushed from the bones with a 10cc syringe and a 30 gauge needle using 1 to 3 ml of DMEM medium (Fisher Scientific, Pittsburgh, PA) containing 10% fetal bovine serum (FBS, Fisher Scientific) under sterile conditions. The bone marrow cells were collected into a 6 cm sterile tissue culture dish (Fisher Scientific) containing 4 ml of the DMEM medium with FBS. The cells were washed twice with 2 ml of sterile phosphate buffered saline (0.137M sodium chloride, 0.0027M potassium chloride, and 0.0119M phosphate buffer) in a 50 ml test tube and pelleted at 500 rpm for 5 minutes in a centrifuge at 4°C. Red blood cells were lysed by incubating cell pellet in 5 ml of modified Gormori's Tris Azo Coupling (TAC) Buffer for 10 minutes at room temperature. The reaction mixture was centrifuged and resulting pellet was suspended in 2 ml of DMEM medium with FBS. After cell counting, 1 to 3 million cells (in 200 µl) was injected intravenously into the tail vein of the irradiated C57BL/6J recipient mice.

This procedure reconstitutes the thymus, spleen, bone marrow and peripheral blood of the recipient mice with EGFP-positive bone marrow-derived cells.

Four weeks after injection, 100 μ l of blood was drawn from recipient mice by cutting the tip of the anesthetized tail and the blood was processed for fluorescence-activated cell sorting (FACS) using the Easy-Lyse Whole Blood Erythrocyte Lysing Kit (Leinco Technologies, St. Louis, MO). In brief, the blood was collected in tubes containing 0.8 ml of 0.5M EDTA to prevent coagulation and 2 ml of 1X Easy lysis buffer was added. The mixture was incubated for 10 minutes at room temperature. After centrifugation at 500 RPM for 2 minutes, the cell pellet was washed twice with 1X FACS washing buffer and cells were fixed in 0.5ml of 1X FACS fixative. The flow cytometry was performed with a Becton-Dickinson (Franklin Lakes, New Jersey) FACScan. Twenty-four chimeric mice showing greater than 60% (range 61 to 90%) EGFP⁺ bone marrow-derived cells were subsequently used in this study.

Haze-generating irregular phototherapeutic keratectomy (PTK) was performed on the chimeric mice in one eye selected at random with a VISX S4IR excimer laser (Abbott Laboratories, Irvine, CA) as previously reported (Mohan, et al., 2008). Briefly, the corneal epithelium was scrapped with a #64 Beaver blade (Becton-Dickinson) and PTK was performed by firing 45 pulses of laser (ablation depth approximately 10 μ m) with a beam diameter of 2 mm on the central cornea, sparing the limbus. The irregularity was generated by positioning a fine mesh screen in the path of laser for the final 50% of the pulses (Mohan, et al., 2008).

2.2. Cornea tissue preparation and immunocytochemistry

At 2 week (3 eyes) or 4 weeks (21 eyes) after PTK, haze formation was gauged with slit lamp biomicroscope and the animals were euthanized. The experimental and contralateral control eyes were removed with 0.12 forceps and Westcott scissors, embedded in liquid OCT compound (Sakura FineTek, Torrance, CA) within a 15 mm X 15 mm X 5 mm mold (Fisher Scientific, Pittsburgh, PA) and snap frozen using previously reported methods (Mohan et al, 2003). The frozen tissue blocks were maintained at -85°C . Tissue sections (7 microns) were cut with a cryostat (HM 505M, Micron GmbH, Walldorf, Germany) and maintained frozen at -85°C until staining was performed.

Double-immunofluorescence staining was performed on experimental and control tissue sections to study the co-expression of EGFP and α -smooth muscle actin (α -SMA) in corneas. The polyclonal rabbit anti-green fluorescent protein antibody (Cat. #AB3080, Millipore, Eugene, OR) was diluted 1:50 in PBS with 1% bovine serum albumin (BSA, Promega, Madison, WI) and placed on the slides for 90 minutes at room temperature. Sections were washed in PBS and the secondary antibody, goat anti-rabbit IgG (H+L) Green, Alexa Fluor 488 (Cat. #A11034, Invitrogen, Carlsbad, CA) was applied at a concentration of 1:100 in PBS for 60 minutes at room temperature. The sections were subsequently incubated for 60 minutes with normal rabbit serum (Cat. # 011-000-120, Jackson ImmunoResearch, West Grove, PN) diluted 1:5 with PBS. After washing again with PBS, a fourth incubation was performed with excess un-conjugated, donkey anti-rabbit IgG (H+L) (Cat. #711-007-003, Jackson ImmunoResearch, West Grove, PN) diluted 1:200 in PBS for 60 minutes at room temperature. Alpha-smooth muscle actin (α -SMA) was detected by incubating tissue sections with a rabbit polyclonal antibody (Cat. # ab5694, Abcam, Cambridge, MA) diluted 1:50 in PBS with 1% BSA for 90 minutes at room temperature. Sections were washed with PBS and then incubated with Alexa Fluor 594 (Cat. # A11037, Invitrogen, Carlsbad, CA) secondary antibody, goat anti-rabbit IgG (H+L) (Red) diluted 1:500 in PBS for 60 minutes at room temperature. Immunocytochemistry controls were performed by omitting one or both primary antibodies.

Coverslips were mounted with Vectashield containing DAPI (Vector Laboratories Inc., Burlingame, CA) to allow visualization of all nuclei in the tissue sections. The sections were

viewed and photographed with a Leica DM5000 microscope equipped with Q-Imaging Retiga 4000RV (Surrey, BC, Canada) camera and ImagePro software.

2.3 Quantification of cells

SMA+ and GFP+ cells were counted real time under the microscope. Counts were made on sections from ten different corneas that were removed at 4 weeks after PTK. In each case, counts of SMA+/GFP+, SMA+/GFP-, S-/GFP+ and S-/GFP- cells in randomly selected, full thickness 400X microscopic fields from the 1) central, 2) mid-peripheral and 3) periphery peri-limbal cornea were performed, as previously described (Mohan et al., 2003).

2.4. Statistical analysis

Statistical comparisons between the groups were performed using analysis of variance (ANOVA) with Student-Newman-Keuls method test, where applicable (Sigma Stat software 3.5). p values less than 0.05 were considered statistically significant.

3. Results

At two weeks following irregular PTK (3 eyes), a few cells in the anterior stroma of the chimeric mice were SMA+GFP+ (data not shown). Few, if any, stromal cells at two weeks after irregular PTK were SMA+GFP-. By four weeks after irregular PTK, many anterior stromal cells in 21 of 21 eyes had cells that were SMA+GFP+ (Fig. 1). A few cells were detected in 3 of 21 four-week irregular PTK chimeric corneas that were SMA+GFP-, but these were rare compared to SMA+GFP+ cells. A higher magnification view of the stroma of the cornea stained for SMA and GFP (Fig. 2) demonstrates the double staining of stromal myofibroblasts for SMA and GFP.

Ten of the chimeric four-week corneas selected at random were subjected to quantitative cell counting (Table). There were 9X more cells that were SMA+GFP+ than were SMA+GFP- in the central, mid-peripheral or peripheral peri-limbal cornea ($P < 0.05$). There were significantly more SMA-GFP+ cells than SMA+GFP+ cells in the central, mid-peripheral and peripheral cornea at 4 weeks after irregular PTK (Table). There was no correlation between the level of chimerization and the percentage of myofibroblasts that were GFP+.

4. Discussion

Myofibroblasts are important contributors to stromal opacity or haze that occurs after corneal injury or surgery (Masur, et al., 1996; Jester, Petroll, and Cavanagh, 1999; Mohan, et al., 2003). After corneal incisional injuries, these cells are active in contracting wounds and preserving the integrity of the cornea. The present study conclusively demonstrates that bone marrow-derived cells can serve as progenitors for the development of corneal myofibroblasts. In the model used in this analysis, at least nine times more myofibroblasts originated from bone marrow-derived precursors than precursors from other sources, presumably cornea. It is even possible that all myofibroblasts were derived from bone marrow-derived cells in this model, since it is not possible to generate chimeras that have more than 95% of bone marrow cells labeled with GFP using the methods in this study. Due to this limitation, it is impossible to exclude the possibility that some of the myofibroblasts generated by the irregular PTK were derived from precursors of corneal origin. Our working hypothesis is that myofibroblasts can be generated from both bone marrow-derived and corneal-derived precursors and that the predominant origin is determined by the type of injury, genetic factors, and perhaps other factors.

Bone marrow-derived cells enter the corneal stroma in high numbers following corneal injuries where the epithelium is wounded (Wilson, et al., 2004). Cytokines and chemokines released from the epithelium and induced in keratocytes serve as chemotactic factors that draw bone marrow-derived cells into the stroma from the limbal blood vessels (Hong, et al., 2001; Stapleton, et al., 2008). Thus, the progenitors that serve as precursor cells for myofibroblast development are attracted into the stroma and directed to undergo differentiation. Further studies are needed to determine whether cytokines such as transforming growth factor beta and platelet-derived growth factor that have been shown to have important roles in directing myofibroblast apoptosis from corneal precursors (Masur, et al., 1996; Jester, et al., 1999; Jester, et al., 2002) are similarly involved in corneal myofibroblast development from bone marrow-derived cells. Similarly, this study does not define the specific lineage of bone marrow-derived cells that serve as precursors for myofibroblasts in the cornea. Further studies are needed to determine if they are monocytes, macrophages, T-cells, or other types of cells.

Earlier attempts by the authors to perform experiments similar to those in this study were hampered by difficulty in generating haze in mice. The irregular PTK technique was developed to facilitate these studies (Mohan, et al., 2008). It is important to demonstrate that bone marrow-derived cells can serve as precursors for corneal myofibroblasts in other species. To this end, rabbits (Hiripi, et al., 2010) and pigs (Kawarasaki, et al., 2009) expressing green fluorescent protein have recently been developed. Both of these species develop severe haze after PRK for high myopia (Mohan, et al., 2003; Sweatt, Ford and Davis, 1999) and, therefore, these results in mice can be explored in either of these species.

Mitomycin C inhibits haze formation after irregular PTK in mice (Wilson, unpublished results, 2009). Therefore, regardless of the origin of the progenitor cells for corneal myofibroblasts, mitomycin C inhibits their generation. If, however, corneal myofibroblasts are also generated from bone marrow-derived cells in humans, new approaches to inhibiting haze generation after photorefractive keratectomy could focus on chemotaxis and development of these precursor cells.

Acknowledgments

This study was supported by EY10056, EY015638, and Research to Prevent Blindness, New York, NY.

References

- Direkze NC, Forbes SJ, Brittan M, Hunt T, Jeffery R, Preston SL, Poulsom R, Hodivala-Dilke K, Alison MR, Wright NA. Multiple organ engraftment by bone-marrow-derived myofibroblasts and fibroblasts in bone-marrow-transplanted mice. *Stem Cells* 2003;21:514–20. [PubMed: 12968105]
- Garana RM, Petroll WM, Chen WT, Herman IM, Barry P, Andrews P, Cavanagh HD, Jester JV. Radial keratotomy. II Role of the myofibroblast in corneal wound contraction. *Invest Ophthalmol Vis Sci* 1992;33:3271–82. [PubMed: 1428702]
- Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest* 2004;113:243–52. [PubMed: 14722616]
- Hiripi L, Negre D, Cosset F, Kvell K, Czömpöly T, Baranyi M, Gócza E, Hoffmann O, Bender B, Böszö Z. Transgenic rabbit production with simian immunodeficiency virus-derived lentiviral vector. *Transgenic Research*. 2010 in press.
- Hong JW, Liu JJ, Lee JS, Mohan RR, Mohan RR, Woods DJ, He YG, Wilson SE. Proinflammatory chemokine induction in keratocytes and inflammatory cell infiltration into the cornea. *Invest Ophthalmol Vis Sci* 2001;42:2795–2803. [PubMed: 11687520]
- Jester JV, Huang J, Barry-Lane PA, Kao WW, Petroll WM, Cavanagh HD. Transforming growth factor (beta)- mediated corneal myofibroblast differentiation requires actin and fibronectin assembly. *Invest Ophthalmol Vis Sci* 1999;40:1959–1967. [PubMed: 10440249]

- Jester JV, Huang J, Petroll WM, Cavanagh HD. TGF beta induced myofibroblast differentiation of rabbit keratocytes requires synergistic TGF beta, PDGF and integrin signaling. *Exp Eye Res* 2002;75:645–657. [PubMed: 12470966]
- Jester JV, Petroll WM, Cavanagh HD. Corneal stromal wound healing in refractive surgery: the role of myofibroblasts. *Prog Retinal Eye Res* 1999a;18:311–356.
- Kawarasaki T, Uchiyama K, Hirao A, Azuma S, Otake M, Shibata M, Tsuchiya S, Enosawa S, Takeuchi K, Konno K, Hakamata Y, Yoshino H, Wakai T, Ookawara S, Tanaka H, Kobayashi E, Murakami T. Profile of new green fluorescent protein transgenic Jinhua pigs as an imaging source. *J Biomed Opt* 2009;14:54017.
- Masur S, Dewal HS, Dinh TT, Erenburg I, Petridou S. Myofibroblasts differentiate from fibroblasts when plated at low density. *Proc Natl Acad Sci USA* 1996;93:4219–4223. [PubMed: 8633044]
- Mohan RR, Hutcheon AE, Choi R, Hong J, Lee J, Mohan RR, Ambrosio R Jr, Zieske JD, Wilson SE. Apoptosis, necrosis, proliferation, and myofibroblast generation in the stroma following LASIK and PRK. *Exp Eye Res* 2003;76:71–87. [PubMed: 12589777]
- Mohan RR, Stapleton WM, Sinha S, Netto MV, Wilson SE. A novel method for generating corneal haze in anterior stroma of the mouse eye with the excimer laser. *Exp Eye Res* 2008;86:235–40. [PubMed: 18068702]
- Netto MV, Mohan RR, Sinha S, Sharma A, Dupps W, Wilson SE. Stromal haze, myofibroblasts, and surface irregularity after PRK. *Exp Eye Res* 2006;82:788–97. [PubMed: 16303127]
- Ogawa M, LaRue AC, Drake CJ. Hematopoietic origin of fibroblasts/myofibroblasts: Its pathophysiologic implications. *Blood* 2006;108:2893–2896. [PubMed: 16840726]
- Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. “Green mice” as a source of ubiquitous green cells. *FEBS Lett* 1997;407:313–9. [PubMed: 9175875]
- Stapleton WM, Chaurasia S, Medeiros FW, Mohan RR, Sinha S, Wilson SE. Topical interleukin-1 receptor antagonist inhibits inflammatory cell infiltration into the cornea. *Exp Eye Res* 2008;86:753–7. [PubMed: 18346730]
- Sweatt AJ, Ford JG, Davis RM. Wound healing following anterior keratectomy and lamellar keratoplasty in the pig. *J Refract Surg* 1999;15:636–47. [PubMed: 10590001]
- Van Parijs L, Rafaei Y, Abbas AK, Baltimore D. Autoimmunity as a consequence of retrovirus-mediated expression of C-FLIP in lymphocytes. *Immunity* 1999;11:763–70. [PubMed: 10626898]
- Wilson SE, Mohan RR, Netto MV, Perez V, Possin D, Huang J, Kwon R, Alekseev A. RANK, RANKL, OPG, and M-CSF expression in stromal cells during corneal wound healing. *Invest Ophthalmol Vis Sci* 2004;45:2201–2211. [PubMed: 15223796]

Fig. 1.

Double immunocytochemistry for SMA and GFP in chimeric mouse corneas with haze at 1 month after irregular PTK. The figure shows the staining results for 3 different corneas (A to C cornea 1, D to F cornea 2 and G to I cornea 3) that had PRK. The overlays are shown in panels A, D, and G. The red stain for SMA in are shown in B, E, and H. The green stain for GFP is shown in C, F, and I. The blue is DAPI staining of cell nuclei. E indicates the epithelium in several panels. In the first cornea (A to C) several myofibroblasts stain for both SMA and GFP (arrows). Several cells in the stroma do not stain for either SMA or GFP (arrowheads). In cornea 2, there are also several cells that stain for both SMA and GFP (arrows). In a third cornea (G to I), the arrowheads indicate the surface of the corneal epithelium. In panels G and

H, arrows indicate stromal cells that stain for SMA, but not for GFP (compare to panel I). In panel I, the arrows indicate cells in the anterior stroma that stain for GFP, but not for SMA (compare to panel H). Panels J to L are control panels in which corneas that had irregular PTK are stained with the primary antibodies for SMA and GFP omitted. Magnification 300X.

Fig. 2.

A high magnification view of a chimeric mouse cornea with haze at 1 month after irregular PTK. In the overlay, high concordance can be seen between the red stain for SMA and the green stain for GFP in several cells in the stroma (arrows). B shows red staining for SMA in these same cells (arrows). C shows green stain for GFP in the same cells (arrows). Blue is DAPI staining of nuclei. Magnification 800X.

Table

CENTRAL CORNEA				
<u>WEEK</u>	<u>TOTAL CELLS DAPI</u>	<u>SMA+/GFP+</u>	<u>SMA+/GFP-</u>	<u>SMA-/GFP+</u>
4 WEEKS	32.2±1.9	0.9±0.3	0.1±0.1*	4.3±0.5 [‡]
MID-PERIPHERAL CORNEA				
<u>WEEK</u>	<u>TOTAL CELLS DAPI</u>	<u>SMA+/GFP+</u>	<u>SMA+/GFP-</u>	<u>SMA-/GFP+</u>
4 WEEKS	36.3±2.3	1.7±0.5	0.1±0.1*	4.1±0.8 [‡]
PERIPHERAL CORNEA				
<u>WEEK</u>	<u>TOTAL CELLS DAPI</u>	<u>SMA+/GFP+</u>	<u>SMA+/GFP-</u>	<u>SMA-/GFP+</u>
4 WEEKS	43.2±2.1	3.7±0.6	0.4±0.3*	5.8±0.9 [‡]

Values represent cells/400x column (n=10) ± standard error

• indicates that the value for SMA+GFP- was significantly different ($P < 0.05$) from the value for SMA+GFP+ in the same zone of the cornea

[‡] indicates that the value for SMA-GFP+ was significantly different ($P < 0.05$) from the value for SMA+GFP+ in the same zone of the cornea