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ORIGINAL PAPER

Mast cells and collagen fibrillogenesis

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Abstract
This article presents 20 combinations of histochemical stainings for the determination of mast cell co-localization with the fibrous component of the connective tissue in the fibrillogenesis course. Best results were obtained using metachromatic detection of mast cells in combination with silver or picro-fuchsin impregnation, staining with brilliant green using van Gieson staining, and a combination of aniline blue staining with neutral red. Proposed variants of histochemical protocols open up new opportunities to analyze the participation of mast cells in extracellular matrix remodeling of the tissue microenvironment in the course of adaptive and pathological processes. Results obtained expand the current theoretical views of the process of fibrillogenesis in the extracellular matrix. They also shed new light on the participation of mast cell secretion components in the molecular mechanisms of fiber formation.

Keywords Mast cells · Specific tissue microenvironment · Collagen fibers · Fibrillogenesis · Skin

Introduction

Aspects of regeneration of extracellular matrix components of the connective tissue have always been in the limelight of researchers. Advanced methodological techniques allowed us to gradually understand new patterns of the integrative-buffer metabolic environment function in both physiological and pathological conditions (Bornstein and Sage 2002; Byers 2000; Hitchcock et al. 2008; Loerakker et al. 2014; Omel'yanenko 2009; Rittie 2017a, b; Rittie et al. 2017). The pioneering works in morphology aimed at the identification of the selective components of the extracellular matrix initiated a large number of studies for the fibers and the amorphous component detection (Buchwalow et al. 2015; Ehrlich, 1878; Lojda et al. 1976; Dabbs 2014; Pearse 1960; Rath 1981; Romeis 2010). This allowed designing numerous histochemical criteria of the extracellular matrix diagnostically significant for the analysis of adaptive processes and the pathological change development assessment. The use of electronic, scanning, and confocal microscopy has opened exceptional opportunities in the knowledge of the structural and physiological features of the connective tissue at the level of supramolecular aggregates (Omel'yanenko 2009; Rittie 2017a; Rittie et al. 2017).

Until now, ongoing work in this direction has provided new data expanding the range of regulatory mechanisms in updating the intercellular matrix components (Bancelin et al. 2014a, b; Ghazanfari et al. 2016; Rittie et al. 2017). Further clarification of tissue cellular composition functions in the amorphous matrix formation and fibrous element state of the specific tissue microenvironment, which is considered to be an important condition for solving these problems. In particular, the role of macrophages in the arrangement of newly formed collagen fibers was discussed (Muldashev et al. 2005). Mast cells (MC), which are distributed practically everywhere in the body and form organ-specific populations, should be considered as one of the promising objects of local homeostasis in the intercellular matrix regulation (Atiakshin et al. 2017; Frossi et al. 2018). It is known that MCs may compose 10% of all immunocompetent cells in the skin (Dwyer et al. 2016). Secretome functional characteristics allow MCs to act as inducers and regulators of the most important physiological and pathological processes—coordination of tissue microenvironment homeostasis, realization of innate and adaptive immunity, initiation and subsequent development of inflammatory and allergic reactions,

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angiogenesis, cell proliferation, and others among them (Galli and Tsai 2008; Kim et al. 2018; Olivera et al. 2018; Wernersson and Pejler 2014). At the same time, a number of secretome components, including proteoglycans, specific proteases, TGF-β, etc. take both direct and indirect part in changes occurring in the structure of the stroma of internal organs, endowing MCs with special potentials in terms of remodeling the intercellular matrix (Atiakshin et al. 2018, 2019; Ronnberg et al. 2012). A frequent association of MC population indices with the intensity of fibrotic changes in the relevant organs suggested their active involvement in the induction of the biogenesis of collagen proteins and extracellular stages of fibrillogenesis (Conti et al. 2018; Hugle 2014; Overed-Sayer et al. 2013; Pincha et al. 2018). This determines the importance of developing and introducing into morphological practice methodological techniques that contribute to specifying the points of application of MC selective effects in the collagen fiber formation, as well as more objective assessment of their contribution to the development of pathological processes with progressive tissue fibrosis.

However, the use of certain histochemical techniques limits the interplay potential assessment between specific connective tissue cells and a fibrous component, which significantly reduces the informative content of visualized structures in relation to both understanding tissue histoarchitectonics and interpreting morphological aspects of the tissue microenvironment fibrous matrix remodeling.

With this study, we present the results of combinations of standard stainings of MC with the stainings of the intercellular matrix fibrous component of the skin connective tissue. These new combination stainings shed new light on the participation of mast cell secretion components in the molecular mechanisms of fiber formation and on the extracellular matrix structure remodeling.

Materials and methods

This study investigated the skin [dorsal neck area at the level of 5–6 cervical vertebrae (C_V–C_{VI})] of Wistar rats of the intact group ($n = 6$) and animals with soft-tissue wounds simulated using excision of the skin and subcutaneous tissue (7 days after modeling the wound process, $n = 6$). Skin biomaterial samples were fixed in buffered 4% formaldehyde and routinely embedded in paraffin. PBS was used for all washings and dilutions. Paraffin tissue sections (5 µm thick) were deparaffinized with xylene and rehydrated with graded ethanols. For pathohistological analysis, tissue sections were routinely stained with H&E. After dehydration and clearing in xylene, the sections were covered with permanent mounting medium and observed on ZEISS Axio Imager.A2 research microscope with the Camera Axiocam 506 color.

Toluidine blue, brilliant green, neutral red, alcian blue stain, periodic acid Schiff reaction, and histochemical detection of chloroacetyl esterase were used to detect MCs (Table 1). In addition, methylene blue, azur I, and polychrome acid fuchsin with aniline blue were used, which are part of the May Grünwald, Giemsa, and picro-Mallory solutions, respectively. Silver impregnation technique, picrofuchsin stain in isolation or using van Gieson protocol stain, aniline blue stain using picro-Mallory technique protocol, fuchsin–resorcinol stain using Weigert's method, and combined alcian blue stain and periodic acid Schiff reaction were used for simultaneous visualization of the intercellular matrix of the connective tissue with MCs (Atiakshin et al. 2017; Buchwalow et al. 2018). Moreover, the identification of MCs is preferably performed after staining the structures of the extracellular matrix. Brilliant green (tetraethyl-4,4-diaminotriphenylmethane oxalate) for histochemical detection of MCs and related elements of the intercellular substance used at a concentration of 1% dye solution. For staining nuclei, Carazzi's hematoxylin was used before staining with brilliant green. Combinations of histochemical techniques used in this study are presented in Table 1. All reagents were purchased from BioVitrum, Moscow, 129626, Russia. Below, we provide more detailed protocols for the combination stainings with toluidine blue and brilliant green.

Combined staining with toluidine blue and silver impregnation

Solutions

1. 0.25% aqueous potassium permanganate.
2. 1% oxalic acid.
3. 5% iron ammonium alum (ferric ammonium sulfate).
4. Ammonia silver: prepare immediately before use.
   - 2 ml of 10% aqueous silver nitrate.
   - 1 ml of 10% aqueous sodium hydroxide or potassium hydroxide.
   - 0.5 ml concentrated ammonia solution.
   - Mix well. Most sediment dissolves in 1–2 min.
5. Formalin solution:
   - 10 ml of concentrated aqueous formaldehyde.
   - 40 ml of distilled water.
   - Prepare before use.
6. 5% aqueous sodium thiosulfate.

Protocol

1. Dewax and bring sections to water. Rinse in distilled water.

Table 1 Histochemical staining combinations

Combinations	Identification of intercellular matrix components	Mast cell detection
1	Silver ammonia solution impregnation protocol	Toluidine blue
2	Picro-fuchsin	Toluidine blue
3	Van Gieson protocol stain (picro-fuchsin)	Toluidine blue
4	Brilliant green[a]	Toluidine blue
5	Aniline blue	Toluidine blue
6	Acid fuchsin	Toluidine blue
7	Eosin	Toluidine blue
8	Van Gieson protocol stain (picro-fuchsin)	Brilliant green
9	Picro-fuchsin	Brilliant green
10	Aniline blue	Brilliant green
11	Eosin	Brilliant green
12	Weigert's method (fuchsin-resorcinol stain)	Neutral red
13	Aniline blue	Neutral red
14	Brilliant green[a]	Neutral red
15	Van Gieson protocol stain (picro-fuchsin)	Neutral red
16	Alcian blue stain + periodic acid Schiff reaction	Alcian blue stain, periodic acid Schiff reaction
17	Gimsa solution (eosin) + May–Grünwald solution (methylene blue)	Giemsa solution (azur I) + May Grünwald solution (methylene blue)
18	Van Gieson protocol stain (picro-fuchsin) + May–Grünwald solution (eosin)	May–Grünwald solution (methylene blue)
19	Picro-fuchsin + May–Grünwald solution (eosin)	May–Grünwald solution (methylene blue)
20	Picro-Mallory staining protocol [polychrome acid fuchsin (acid fuchsin + orange G), aniline blue]	Picro-Mallory staining protocol [polychrome acid fuchsin (acid fuchsin + orange G), aniline blue]

[a]Brilliant green can be used to stain mast cells and fibrous elements of the connective tissue

2. Potassium permanganate—5 min. Rinse in distilled water for 1 min.
3. Decolorize with oxalic acid (3 min). Rinse with distilled water.
4. Treat with ammonium alum for 5 min. Rinse in four shifts of tap water for 3 min each.
5. Rinse the slides in distilled water for 2 × 3 min.
6. Ammonia silver for 5 min.
7. Rinse quickly in distilled water (one or two dips).
8. Formalin solution for 1 min.
9. Rinse in distilled water for 2 × 3 min.
10. Sodium thiosulfate for 1 min.
11. Rinse in distilled water.
12. Stain mast cells with toluidine blue (0.1% aqueous solution) for 5 min.
13. Rinse quickly in 99% isopropyl alcohol.
14. Coverslip with permanent mounting medium.

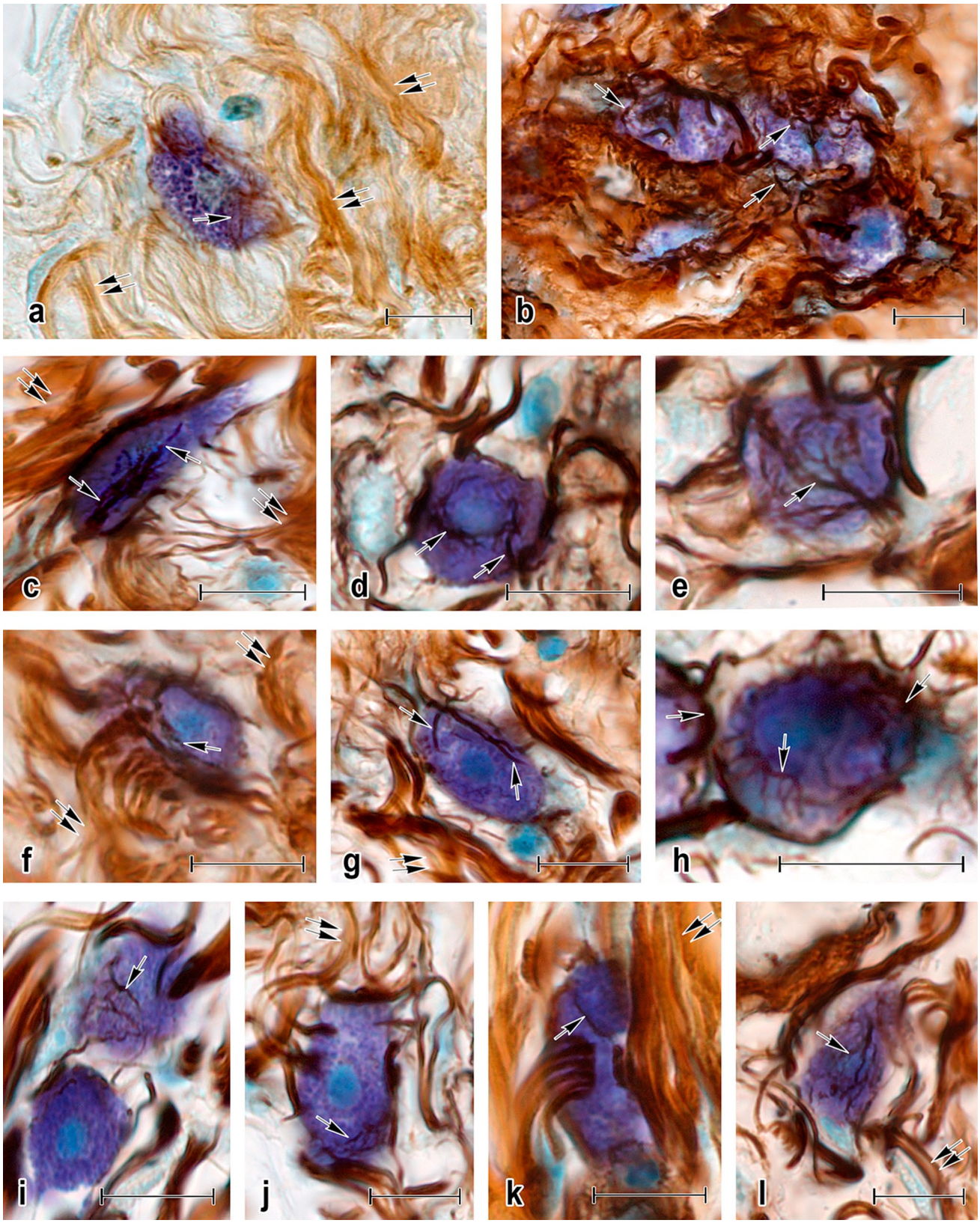
Combined staining with toluidine blue and picro-fuchsin

Solutions

1. Picro-fuchsin solution: 5–10 ml of a 1% aqueous solution of acid fuchsin is added to 100 ml of a saturated aqueous solution of picric acid.
2. 0.1% aqueous solution of toluidine blue.

Protocol

1. Dewaxing sections. Rinse in distilled water.
2. Staining with picro-fuchsin solution for 5 min.
3. Rinse with tap water for 2 min.
4. Staining with 0.1% aqueous toluidine blue solution for 5 min.
5. Rinse quickly in 99% isopropyl alcohol.
6. Coverslip with permanent mounting medium.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◂**Fig. 1** Rat skin. A combination of silver impregnation with toluidine blue staining. **a** Normal, **b–l** wound healing process at various stages after skin damage. **a** The prevalence of mature collagen fiber bundles (indicated by a double arrow); the fiber formation low intensity in the field of extracellular matrix co-localization with the MC plasmalemma (indicated by an arrow). **b** High content of impregnated fibrous component around the MC (indicated by an arrow). **c–l** Variants of the reticular fiber localization of various caliber with MC (indicated by an arrow). The thinnest fibers extending from the MC and forming the network are located paracellularly. The paracellular foci of fiber formation in the form of an annular reticular network (**d**, **e**, **h**) are especially noteworthy. Reticular fibers can pass into mature thicker collagen fibers (indicated by a double arrow). Bar 10 µm

Combined staining with brilliant green and van Gieson's stain

Solutions

1. To prepare a 1% solution of brilliant green, dissolve 1 g brilliant green in 52 ml 95% ethyl alcohol, add distilled water to 100 ml volume, and filter.
2. Weigert's hematoxylin is prepared immediately before staining, mixing equal volumes of Weigert basic solutions (A and B), which are stored separately.

- Weigert solution (A): 1 g hematoxylin is dissolved in 100 ml of 96% ethanol;
- Weigert solution (B): 4 ml of a 29% aqueous solution of ferric chloride is mixed with 1 ml of concentrated hydrochloric acid and 95 ml of distilled water is added. When mixing the solutions, the second solution is poured into the first in a somewhat smaller amount, and then, the second solution is added to the resulting mixture dropwise from the pipette to an equal amount with the first solution.

3. Picro-fuchsin solution: 5–10 ml of a 1% aqueous solution of acid fuchsin is added to 100 ml of a saturated aqueous solution of picric acid.

Protocol

1. Dewax sections and bring to the water.
2. Apply Wiegert's hematoxylin for 5 min.
3. Rinse with tap water for 5 min, then in distilled water for 5 min.
4. Stain with picro-fuchsin solution for 5 min.
5. Apply four drops of 1% brilliant green solution to a slide for 40 s.
6. Immerse in 99% isopropyl alcohol, differentiate for 5–15 min under microscope control.
7. Coverslip with permanent mounting medium.

Combined staining with brilliant green and picro-fuchsin

Solutions

1. 1% brilliant green solution (preparation see above).
2. Picro-fuchsin solution (preparation see above).

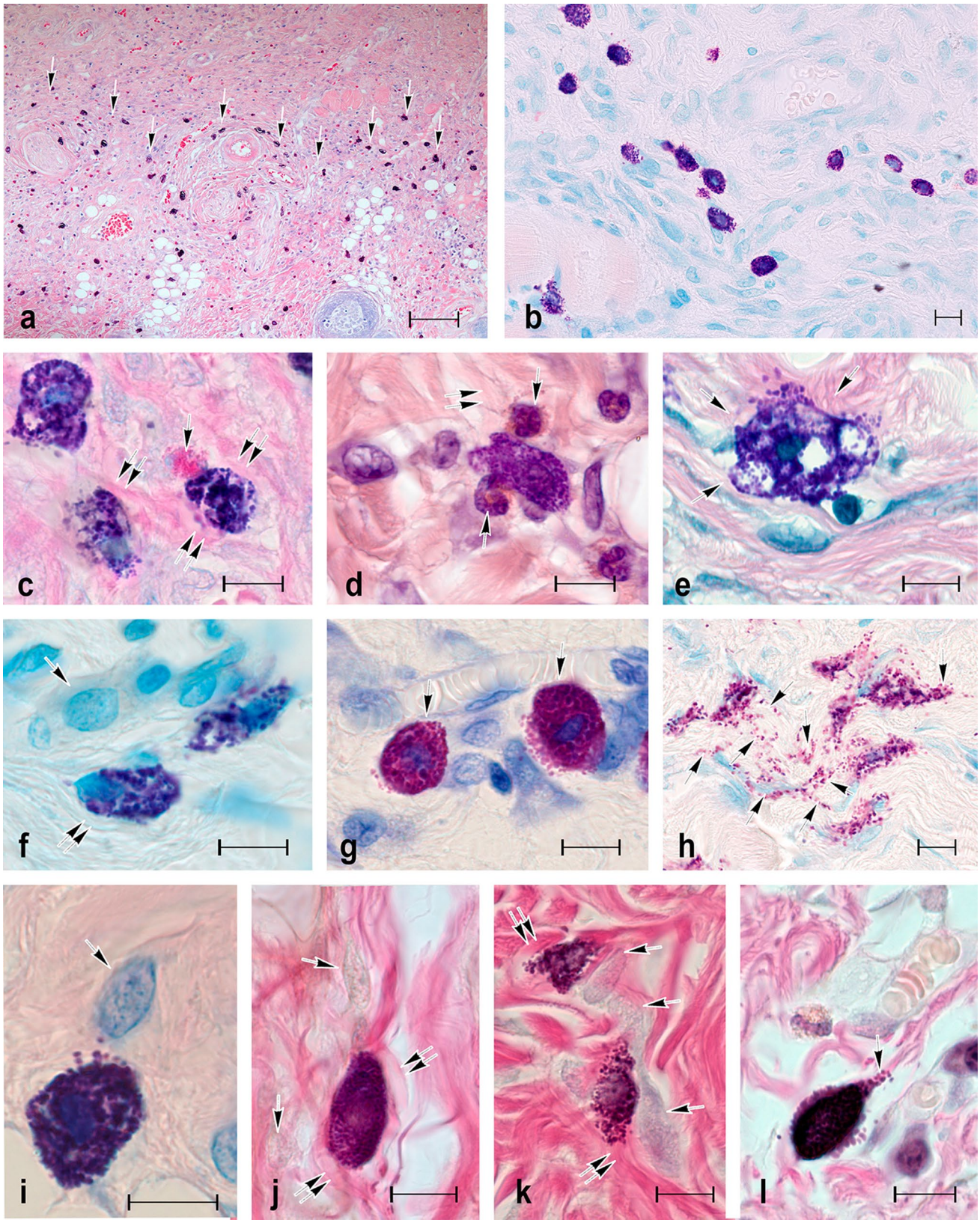
Protocol

1. Dewaxing sections and bring to the water.
2. Apply 4 drops of 1% brilliant green solution on the slide for 40 s, then quickly immerse the slide in 99% isopropyl alcohol and differentiate for 5–15 min under microscope control.
3. Staining with picro-fuchsin solution for 5 min.
4. Rinse quickly in distilled water.
5. Quickly dehydrated in 99% isopropyl alcohol.
6. Coverslip with permanent mounting medium.

Results

Toluidine blue

There are cases where isolated staining with toluidine blue allows differentiating mast cells and the surrounding stroma (Fig. 2f); however, the almost complete absence of its contrast makes it challenging to detect the fibrous component of the intercellular substance. The combination of toluidine blue staining with impregnation with silver has demonstrated the successful preservation of metachromatic staining of MCs (Figs. 1, 5c, g). Identification of the fibrous component of the dermis is accompanied by selective contrasting of thin reticular or pre-collagen fibers stained in black, while thicker and more mature collagen fibers acquire different shades of brown–yellow color (Figs. 1, 5c, g). The degree of effectiveness of using this method to assess the co-localization of collagen fibers with MCs depends on the glycosaminoglycans of their secretome, which determine the intensity of metachromasia. In particular, this combination appeared to be more successful in the study of laboratory animals with skin wound simulation compared to intact rats (Fig. 1). MC metachromasia allowed identifying their adherence to reticular fibers containing type III collagen. The frequency of MC contacts with impregnated fibers was numerous and may represent a separate task of morphological analysis when studying the characteristics of the skin MC population. Part of the MC was freely located in the intercellular matrix, while the other lay adjacent to the impregnated fibers (Fig. 1f, g, k), sometimes forming a pronounced network (Figs. 1c–e, h, i, 5c). Attention was drawn to the fact that on some MCs, there are thin fibers that were in contact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◂**Fig. 2** Rat skin after modeling the wound process. **a**–**f** Staining with toluidine blue separately (**f**) or in combination with picro-fuchsin (**a**), Giemsa stain (**b**–**d**) and van Gieson's stain (**e**), **g**–**l** staining with May–Grünwald solution in isolation (**g**–**i**) or in combination with van Gieson's stain (**j**), picro-fuchsin (**k**), and Giemsa stain (**l**). **a** A general view of mast cells and the fibrous component of the intercellular matrix of the skin connective tissue. The border (arrow) of the dermis with a high content of MC (at the bottom, in the direction of the epidermis) and the zone of their absence is well visualized. Collagen fibers are colored red. **b** Predominant accumulation of MCs in a limited area of the dermis. **c**, **d** Contacts of MCs with neutrophils (indicated by arrows) surrounded by bundles of collagen fibers (indicated by double arrows). **e** Actively secreting MCs surrounded by collagen fibers (indicated by arrows). **f** MC localization with fibroblast (indicated by an arrow) and fibers (double arrow). **g** Adherence of MCs to the basal membrane of the capillary (indicated by arrows) and partial degranulation. **h** Exocytosis of secretory granules of MCs in localized dermis loci (indicated by arrows). **i**–**k** Options for MC contacting with fibroblasts (indicated by arrows) and collagen fibers (indicated by double arrows). **l** Secretion of MC granules in the region of collagen fiber (indicated by an arrow) and paracellular space. Bar = 10 µm for the entire layout and 100 µm for the (**a**)

with the surface of the plasmalemma or which started at the microlocus of their adjacency to the cell and continued into the intercellular matrix of the tissue microenvironment (Figs. 1c, g, i, j, l, 5c, g). Similar patterns were observed when MCs of isolated multicellular groups were formed; radially and tangentially oriented thin impregnated fibers departed from the plasmalemma of these MCs (Fig. 1b). In addition, the MC degranulation can be observed through the exocytosis mechanism in the plasmalemma area aside from the contact with fibers. MCs were sometimes identified, plainly mounted in the framework of the reticular fibers, covering the plasmalemma from all sides (Fig. 1k). The thinnest impregnated structures on the cell surface possibly reflect the morphological manifestations of initial stages of the fibril formation from tropocollagen macrocomplexes in the intercellular matrix. The frequency of MC co-localization with impregnated fibrous elements and fibroblasts, as well as the content of impregnated fibers, increased in the skin of animals with wound simulation compared to the animals of the intact group (Figs. 1, 5).

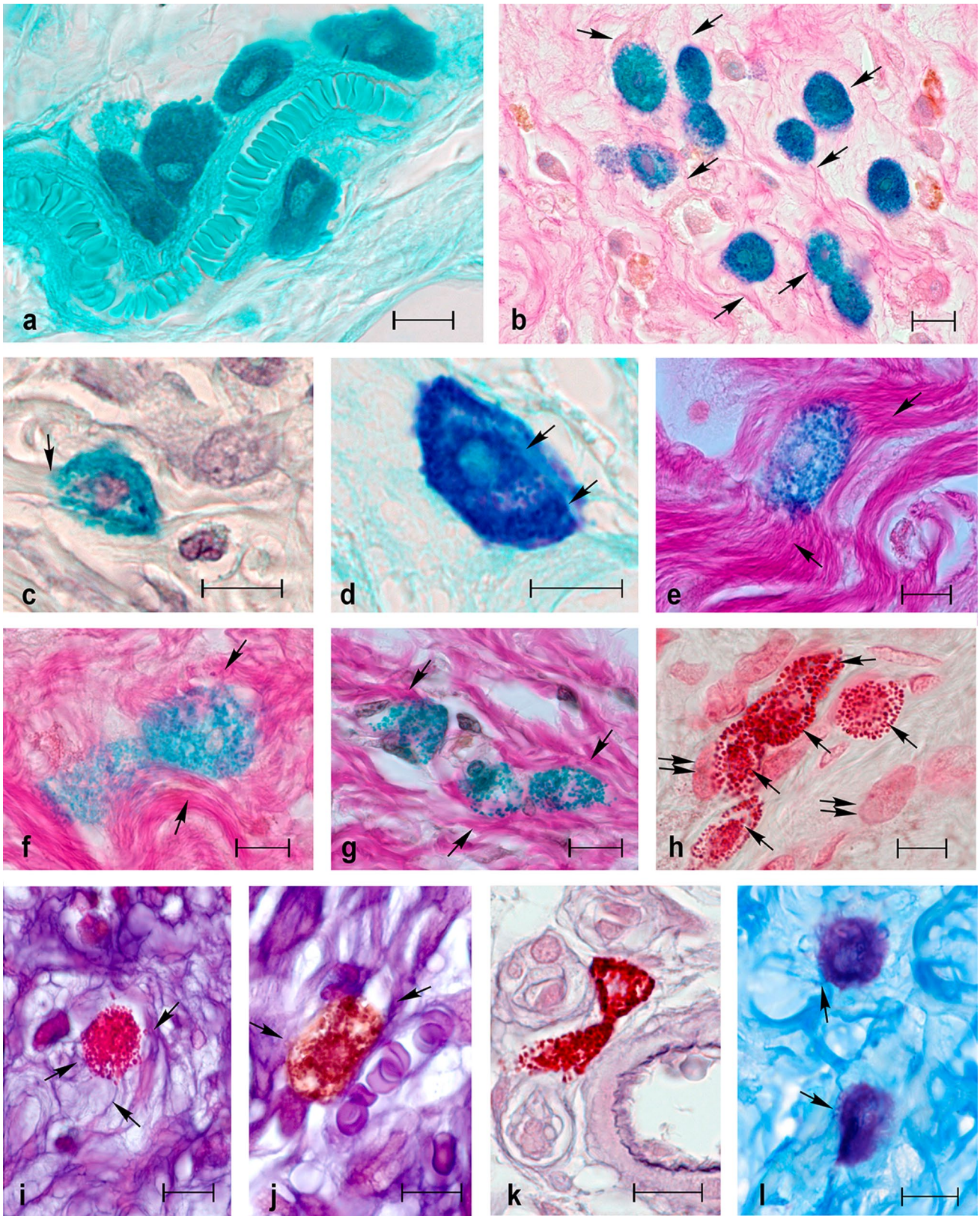
Staining with toluidine blue and picro-fuchsin appeared to be a suitable combination due to its achievement of the simultaneous effect of collagen fiber identification in red shades and MC identification in various shades of purple (Fig. 2a). Furthermore, toluidine blue independently stained the nuclei of cells; this allowed simultaneously visualizing MCs not only with collagen fibers but also with the nuclei of other cellular representatives of the tissue microenvironment. This fact provides a means of evaluation for MC co-localization with fibroblasts (Fig. 5n). It should be noted that MCs were more frequently adjacent to fibroblasts in the skin of animals with wound simulation, which makes this characteristic significant for interpreting the activity of intercellular matrix remodeling. The completion of van Gieson protocol stain when contrasting the nuclei with iron hematoxylin in combination with toluidine blue also provided a successful visualization of MC and collagen fibers (Fig. 2e). During secretion, MC granules were sometimes localized in the connective tissue; this fact enabled the assumed initiation of fibril formation routing in the skin dermis (Fig. 2e). The combination of toluidine blue with aniline blue provided good potential for the MC detection in the intercellular matrix elements (Fig. 3l). However, despite the obvious metachromasia of the MC cytoplasm, this combination should not be considered the method of choice compared to the other protocols, due to fibrous elements poor contrasting with aniline blue, as well as MC granular component poor visualization (Fig. 3l). In addition to a clear visualization of metachromatic mast cells, the combination of toluidine blue and Giemsa solution staining allows successfully identifying collagen fibers, and granulocytes, including neutrophils and fibroblasts (Figs. 2b–d, 5e).

Combined staining with acid fuchsin was characterized by excessive contrasting of the intercellular matrix; however, it was acceptable for visualization of MC co-localization with fibroblasts or fibrocytes. The least suitable was combined staining of the slides with toluidine blue and eosin. It might be recommended to apply Puebla-Osorio et al. protocol (2017) to increase the effectiveness of this combination.

Thus, the high content of glycosaminoglycans in mast cell granules preserves the metachromatic properties of the secretome when combining toluidine blue staining with other dyes that are selective for extracellular matrix fibers. However, the clearest visualization of mast cells with collagen fibers is achieved by simultaneous impregnation with silver or picro-fuchsin staining. In view of identifying the initial stages of fibrillogenesis, the advantages of using the silver impregnation protocol become apparent.

Methylene blue

The use of methylene blue as a part of May Grünwald solution showed the MCs effective detection against the background of weakly colored pink collagen fibers; their contrasting is achieved partly due to the eosin presence in the stain composition (Figs. 2g–i, 5o). Better detection of collagen fibers was provided by the combination of van Gieson's stain (Fig. 2j), picro-fuchsin (Fig. 2k), and Giemsa stain (Fig. 2l). In addition, adjacent cells nuclei contrasting of a specific tissue microenvironment also allows from one degree to another concluding on MC co-localization with fibroblasts or fibrocytes (Figs. 2i–k, 5b, l). The combination with Giemsa stain together with adequate contrasting of the extracellular matrix may result in increased staining of mast cell secretion; this fact must be taken into account when staining (Fig. 2l). It is worth noting that the isolated Giemsa stain may also be applied to detect extracellular matrix with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◂**Fig. 3** Rat skin after modeling the wound process. **a**–**g** Staining with brilliant green in isolation (**a**) and in combination with staining with picro-fuchsin and Mayer's hematoxylin (**b**), Mayer's hematoxylin (**c**), toluidine blue (**d**), picro-fuchsin (**e**, **f**), van Gieson's stain (**g**), **h**–**k** staining with neutral red in isolation (**h**), in combination with aniline blue (**i**, **j**), Weigert's stain (express method) (**k**), and **l** combined staining with aniline blue and toluidine blue. **a** Perivasal location of the MC. **b** A group of MCs localized with thin collagen fibers (indicated by arrows). **c** Close contact of MC with a bundle of collagen fibers (indicated by an arrow). **d** Intracytoplasmic localization of collagen fiber in the MC (indicated by arrows). **e**–**g** MC localization with the fibrous component of the skin dermis (indicated by arrows). **h** MC (indicated by arrows) are localized near fibroblasts (indicated by double arrows), **i**, **j** Various options for the MC co-localization with collagen fibers (indicated by arrows). **k** Two contacting mast cells and an arteriole, in which the inner elastic membrane is clearly visible. **l** Mast cell co-localization with the fibrous component of the extracellular matrix of the dermis, exocytosis of granules with a parafibrillar arrangement (indicated by arrows). Bar 10 µm

mast cells due to azur I being one of its components and to visualize the nuclei of other cells of the specific tissue microenvironment, including fibroblasts.

Therefore, according to the results of the analysis of the effectiveness of using methylene blue to study mast cell co-localization with the fibrous component of connective tissue, it is effective only if it is used in combination with other solutions, e.g., May–Grunwald. This allows us to consider the use of methylene blue less convenient than toluidine blue when combined with fiber-selective dyes.

Brilliant green

As for results of staining with brilliant green in combination with other histochemical protocols, it should be noted that it is considered to be highly effective for visualization of the skin MCs (Fig. 3a). When contrasting the nuclei with Mayer's hematoxylin, MCs are well visualized in turquoise blue shades, the number of which per unit of the tissue area is comparable to the results of staining with toluidine blue (Figs. 3c, 5a, d). The application of picro-fuchsin with brilliant green combination allows successfully contrasting MCs against the background of collagen fibers stained red (Fig. 3b). Since brilliant green has a weak affinity for nuclear structures and is quickly washed out during differentiation, it should be better combined with classical van Gieson protocol stain, in which the nuclei are stained with Weigert's hematoxylin (Fig. 3g). However, after combination with picro-fuchsin nuclei contours, visualization of other cells becomes possible without counterstaining thorough microscopic examination (Fig. 3e, f). The tropism of brilliant green to the structures of the fibrous component of the intercellular matrix creates methodological conditions for its combination with toluidine blue and provides additional information when assessing the MC co-localization with fibers (Fig. 3d). In addition, this combination can provide information on the qualitative composition of the secretome, since there have been found various affinities of its components for stains. On the other hand, the aniline blue with brilliant green combination resulted in difficulties in detecting turquoise MCs on the blue-colored structures of the intercellular matrix; the fact prevents this protocol from being applicable in histochemical practice. Similarly, staining with brilliant green was not effective in combination with eosin, causing non-selective masking diffuse staining of skin structures in pink shades.

Summarizing the above, it should be noted that for the study of mast cells and fibrillogenesis in the skin using brilliant green, it is most effective to combine this staining with the van Gieson protocol, in which not only the fibrous component of the extracellular matrix is selectively detected, but also the nuclei of the cells are contrasted.

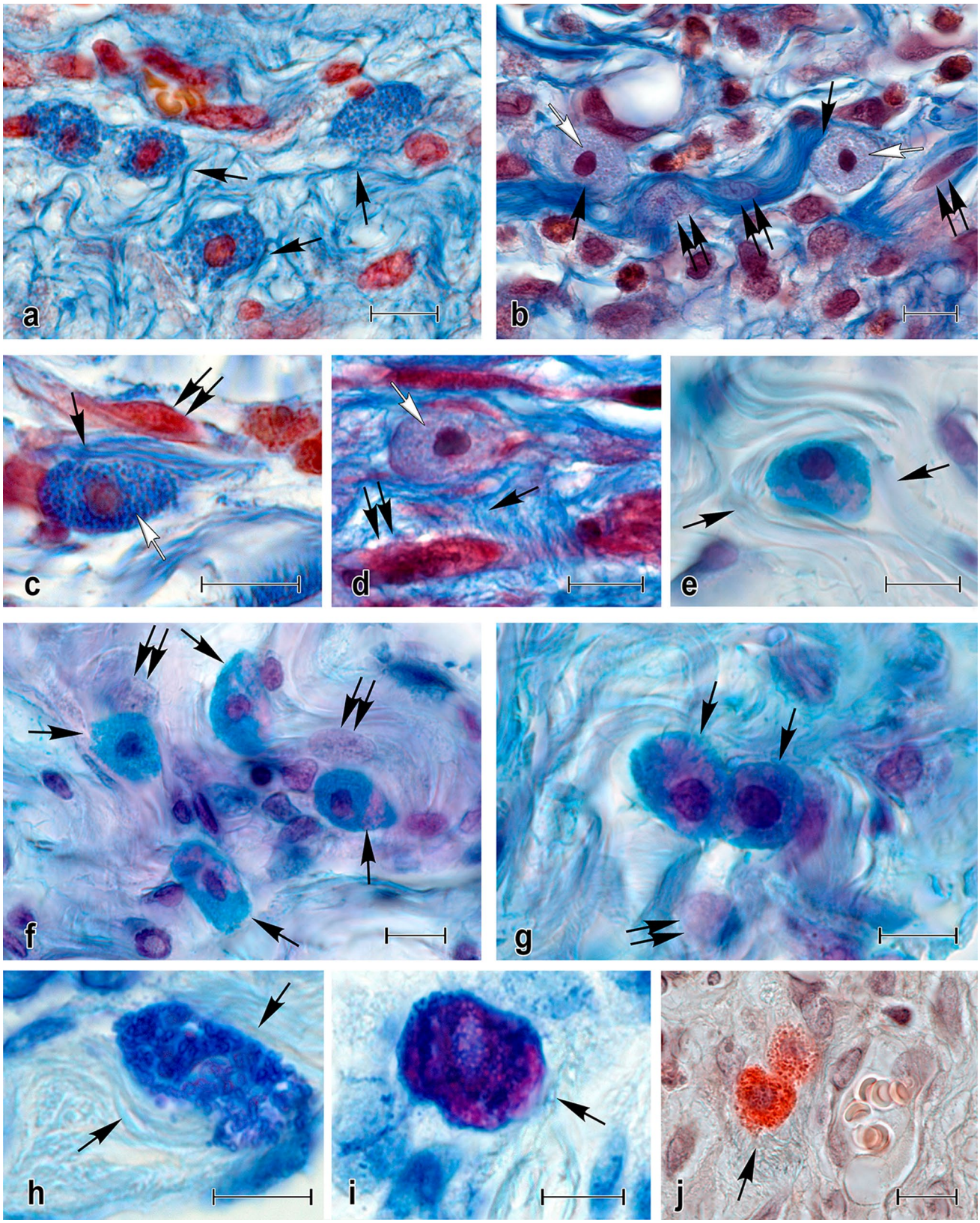
Neutral red

The use of neutral red as an independent reagent in MC detection is not convenient enough due to its ability to contrast cell nuclei, which can mask the visualization of granules stained in similar color shades. However, in some cases, this allows observing MC co-localization with fibroblasts (Fig. 3h). The combination with aniline blue stain has proved itself to be quite informative, allowing the evaluation of fiber co-localization with the skin MCs (Fig. 3i, j). It is advisable to avoid excessive staining of skin sections with aniline blue since these results in the appearance of the difficult-to-differential background. Weigert's hematoxylin and neutral red stain combination allow successful visualizing of MCs and elastic fibers (Fig. 3k). The content of elastic fibers in the skin dermis is low compared to the content of collagen fibers; despite this fact, certain MCs come into contact with them. When completing an individual Weigert's hematoxylin staining, MCs are also visualized in dark violet shades; however, staining with neutral red can significantly improve the quality of their detection. The neutral red and brilliant green combination results in the dark staining of MCs due to the combined application of stains on the secretory material.

Thus, the most advantageous combination of neutral red for studying the participation of mast cells in fibrillogenesis is achieved by combining staining with aniline blue, which allows for advantage of the staining of cellular and fibrous structures in different colors.

Aniline blue

The application of aniline blue in combination with polychrome acid fuchsin (the picro-Mallory staining protocol) led to a clear visualization of collagen fibers, and, when stained with MCs, their co-localization with extracellular matrix elements became clearly noticeable (Fig. 4a–d).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◂**Fig. 4** Rat skin after modeling the wound process. **a**–**d** Picro-Mallory staining, **e**–**g** combined staining with alcian blue (pH 2.5) and periodic acid Schiff reaction, **h**, **i** staining with aniline blue and Giemsa solution, and **j** detection of chloroacetyl esterase activity. **a** MC localization at the site of active fiber formation. Collagen fibers that do not form bundles are determined (indicated by arrows). **b** MCs (white arrows) are localized with a large bundle of collagen fibers (indicated by black arrows) and fibroblasts (indicated by double black arrows) are determined. **c**, **d** Various options for the location of collagen fibers (indicated by an arrow) between MCs (arrows) and fibroblasts or myofibroblasts (indicated by a double arrow). **e** MC co-localization with a bundle of collagen fibers of the dermis (indicated by arrows). **f**–**g** The formation of the group of MCs (arrows) and fibroblasts (indicated by double arrows) within a limited field of the dermis surrounded by collagen fibers, the interaction of MC (**g**). **h**–**i** Options for MC contacting with the fibrous component (indicated by arrows) of the extracellular matrix of the skin. **j** Collagen fibers (indicated by an arrow) close to MC localization. Bar 10 µm

At the same time, the detection of contractile proteins including smooth myocytes appears to be a beneficial advantage of this technique; this fact allows identifying myofibroblasts in the specific tissue microenvironment due to the red shades of the cytoplasm (Fig. 5j, k). This technique gives the opportunity to observe the beginning of fibroblast transformation into myofibroblast (Fig. 5i).

The application of aniline blue combination with a special Giemsa solution led to unexpected results. The fibrous component was stained diffusely, while Azur 1 from Giemsa's reagent resulted in the metachromatic detection of mast cells. As a result, mast cells could be identified against a bluish background containing fibers (Fig. 4i). However, mast cell granules looked unusual in some cases: the metachromatic core of the granule was not detected, and the outer granule contours became apparent, often turning into one another forming numerous intracellular passages (Fig. 4h).

Reasonably, the greatest advantage of the picro-Mallory protocol is the ability to indirectly judge the presence of myofibroblasts in the studied focus. At the same time, the successful detection of mast cells will obviously be achieved only if they have high functional activity and a high content of glycosaminoglycans in granules.

Alcian blue

The application of alcian blue in combination with periodic acid Schiff reaction was quite successful in detecting MC co-localization with the connective tissue fibers (Fig. 4e–g). In addition to the unequal staining of the granular component of mast cells in blue or pink shades depending on the degree of biopolymer sulphation (Atiakshin et al. 2017), nearby fibers became well-contoured (Fig. 4e–g).

Discussion

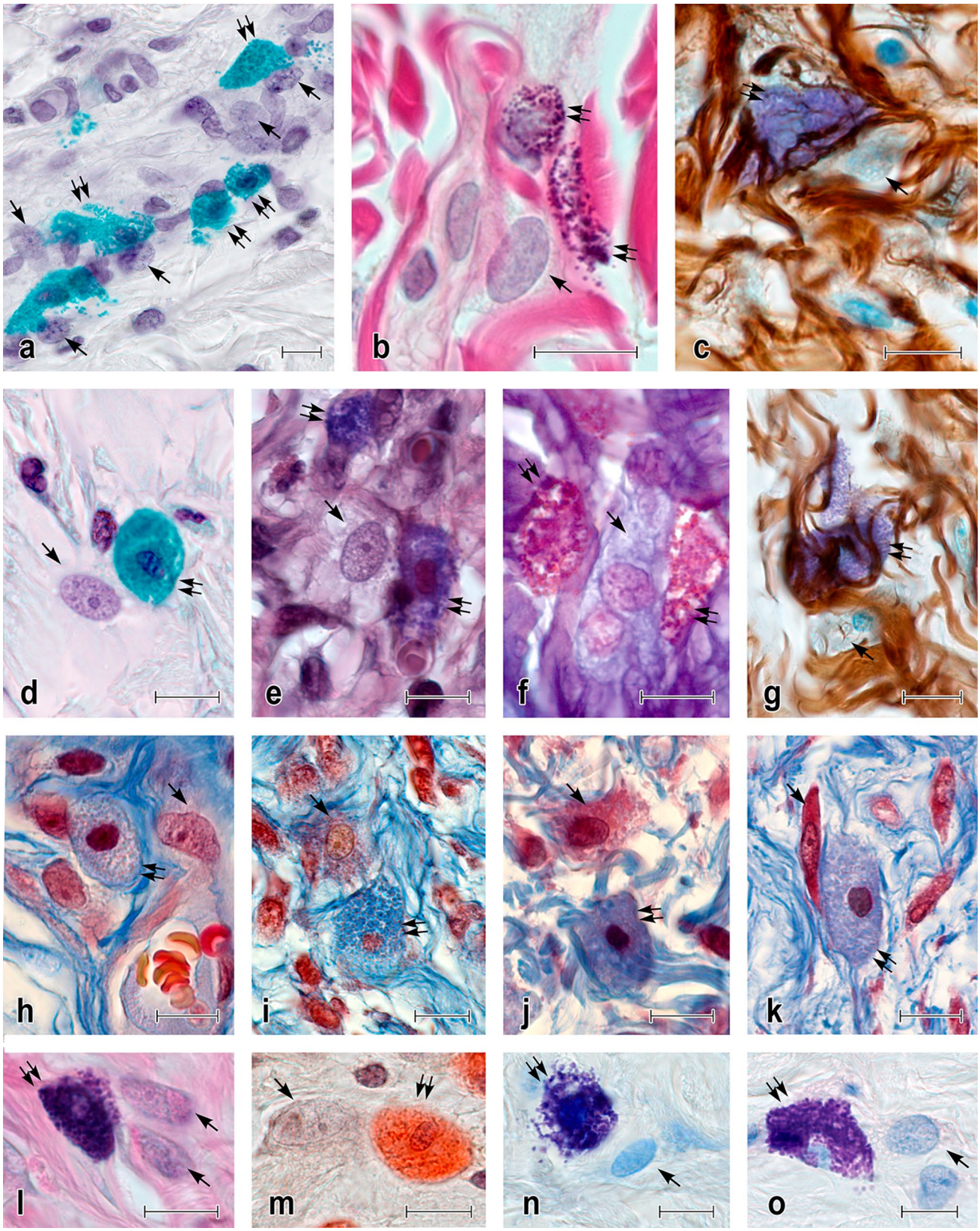
The properties of mast cells enable the use of classic histochemical protocols for the effective selective double staining of the metachromatic components of the secretome and dermis fibers. This provides researchers with unique data to interpret the state of fibrillogenesis and the secretory activity of mast cells under normal and pathological conditions. Moreover, among the studied 20 different combinations of histochemical techniques, the most convenient for the formulation and further interpretation of the obtained results includes metachromatic detection of mast cells with toluidine blue in combination with silver impregnation or picro-fuchsin, staining with brilliant green using the van Gieson technique or picro-fuchsin, as well as aniline blue with neutral red. It should also be noted that silver impregnation allows us to study the initial stages of fibrillogenesis in the dermis of the skin, while other combinations provide a more static presentation of the existing ratio of collagen fibers and mast cells. Thus, histochemical analysis even without supplementing the study with more complex molecular morphological methods allows us to reveal new mechanisms of the formation of fibrosis in various organs.

Many researchers have noticed an increase in mast cell numbers with the development of fibrosis of various organs (Lombardo et al. 2019; Shimbori et al. 2019). This fact appears to be indirect evidence of MC participation in pathological processes accompanied by excessive formation of intercellular matrix components, including the fibrous component.

The applied histochemical protocols obtained results aimed at the simultaneous detection of MCs and the extracellular matrix indicate both the possibility of the direct MC inductive effect on the increase in the collagen proteins production in fibroblasts and myofibroblast and the active MC participation in the extracellular assembly process of collagen fibers from tropocollagen, microfibrillar, and fibrillar subunits (Fig. 6).

The beginning of fiber molecular subunits biogenesis occurs intracellularly (primarily in fibroblasts, fibrocytes, or myofibroblast) (Agarwal et al. 2019; Fridman and Agarwal 2019; Omel'yanenko 2009). Further processing of collagen proteins involves the collagen transfer from the endoplasmic reticulum to the Golgi complex, and then, they are secreted into the intercellular matrix. However, there are some studies that evidence the possibility of microfibrils' intracellular formation within the granulation tissue during collagen protein hyperproduction (Ina et al. 2005).

As a result of the complete processing, the collagen molecule acquires one large central triple-helical domain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◂**Fig. 5** Mast cell co-localization with fibroblasts in the rat skin after modeling the wound process. **a**, **d** Staining with brilliant green and Mayer's hematoxylin (**a**, **d**), **b**, **l** staining with May–Grünwald solution in combination with picro-fuchsin, **c**, **g** impregnation with silver staining with toluidine blue, **e** staining with Giemsa solution with toluidine blue, **f** staining with aniline blue and neutral red, **h**–**k** staining according to picro-Mallory staining protocol, **m** identification of the activity of chloroacetyl esterase, **n** staining with toluidine blue, and **o** staining with May–Grünwald solution. Different variants of fibroblast co-localization (indicated by arrows) with MC (indicated by double arrows). **a** Group of fibroblasts is localized with several MCs forming a functional cluster of fibrillogenesis in the skin dermis. **h**–**k** Histochemical manifestations of the successive transformation of fibroblast (**h**) into myofibroblast (**i**–**k**) with the accumulation of contractile elements in the cytoplasm. Bar 10 µm

and terminal short non-collagenous sequences called telopeptides and becomes capable of further self-organization into a microfibril (Fig. 6) (Mienaltowski and Birk 2014). In the extracellular matrix, the macromolecular complexes of fibril-forming collagen are sequentially assembled from monomeric subunits with further linear and lateral fiber growth (Fig. 6) (Wu and Li 2015). In the peri-plasmalemmal emerging nucleation sites of the fibroblast or myofibroblast, collagens of type V, XI, and III are involved. The first stages of collagen maturation outside the fibroblast cytoplasm or myofibroblast for fibrillogenesis are associated with the cleavage of amino- and carboxy-terminal propeptides and the tropocollagen formation using several enzymes involved (Kessler et al. 1996; Scott et al. 1999; Colige et al. 2002; Mienaltowski and Birk 2014) (Fig. 6). Enzymes that process a particular propeptide have specificity for each individual type of collagen. In this aspect, the ability of chymase to enzymatically cleave a C-propeptide from type I collagen molecules is earlier shown to be of great importance (Kofford et al. 1997).

The primary aggregate combination of tropocollagen molecules is a reference point in the collagen fibril formation that may contain different types of macromolecules. Type III collagen is localized on the surface of fibrils represented by type I collagen (Omel'yanenko 2009) and can serve as an initiation point (nucleator) for further formation of fibrils. Type V collagen enters the central regions of the fibril, and is responsible for regulating fiber growth in the lateral direction, determining thickening processes (Birk et al. 1990a, b). Moreover, nonfibrillar collagen molecules must be present on the surface of fibrils, which are necessary for organizing interaction with other structural elements of the intercellular matrix (Ghazanfari et al. 2016) and small proteoglycans (decorin, biglycan, fibromodulin, etc.) (Omel'yanenko 2009). Thus, the points of various components' possible inductive effect of MC secretome on the self-organization process of collagen fibers in the intercellular matrix became apparent. In this aspect, it is important to consider the MCs interaction with fibroblasts, fibrocytes, or myofibroblasts—the collagen proteins, main producers in a specific tissue microenvironment.

"Mast cell: fibroblast" axis in fibrillogenesis

It is well established that fibroblasts maintain human MC survival. Convincing morphological evidence of the MC co-localization with fibroblasts was obtained in the performed study (Fig. 5). This fact may appear to be one of the structural criteria for assessing the MCs regulatory effect on myofibroblasts or fibroblasts in terms of the collagen biogenesis and other proteins of the intercellular matrix amorphous component.

It is known that MC produces several mediators having the ability to selectively increase the transcriptional activity of fibroblasts (Artuc et al. 2002; Garbuzenko et al. 2004) (Fig. 6). Moreover, in this mechanism, direct contact of MCs with fibroblasts, which, as shown by several authors, is necessary for the activation of the latter, acquires special significance (Koma et al. 2005) (Fig. 6).

One of the mast cell chemoattractants and the mediator of their adhesion to fibroblasts is the plasminogen activator inhibitor-1 (PAI1) (Bhattacharyya et al. 2016; Pincha et al. 2018). A number of cell surface proteins on fibroblasts mediate the interaction between fibroblasts and mast cells. These include membrane-bound stem cell factor (Hogaboam et al. 1998), hyaluronic acid receptors (Termei et al. 2013), fibrinogen (Oki et al. 2006), and gap junctions (Au et al. 2007).

The attachment of mast cells to fibroblasts leads to the activation of both cell types in a time sequence. A quick response to this interaction is an increase in the expression of alpha-smooth muscle actin (α-SMA) in fibroblast and a degranulated MC phenotype (Pincha et al. 2018). In turn, degranulated mast cells are sources of fibroblast activators such as histamine, tryptase, chymase, interleukins, and TGF-β (Overed-Sayer et al. 2013; Theoharides et al. 2012). The subsequent result of this heterotypic interaction is the production of IL-4 and IL-13 pro-fibrotic interleukins in activated MCs that can induce fibroblast proliferation (Hashimoto et al. 2001; Kaviratne et al. 2004).

Extracellular stages of fibrillogenesis: a target for MC secretome

MCs are closely involved in tissue morphogenesis due to their participation in the formation of intercellular substance. Collagen fibrillogenesis is understood as a process strictly controlled by cells and localized near the plasma membrane (Kadler et al. 2008). The Spatio-temporal regulation of molecular combinations during packaging and transportation to secretion sites is provided by a mechanism that makes this process adequate to the corresponding challenges of the tissue microenvironment. Extracellular

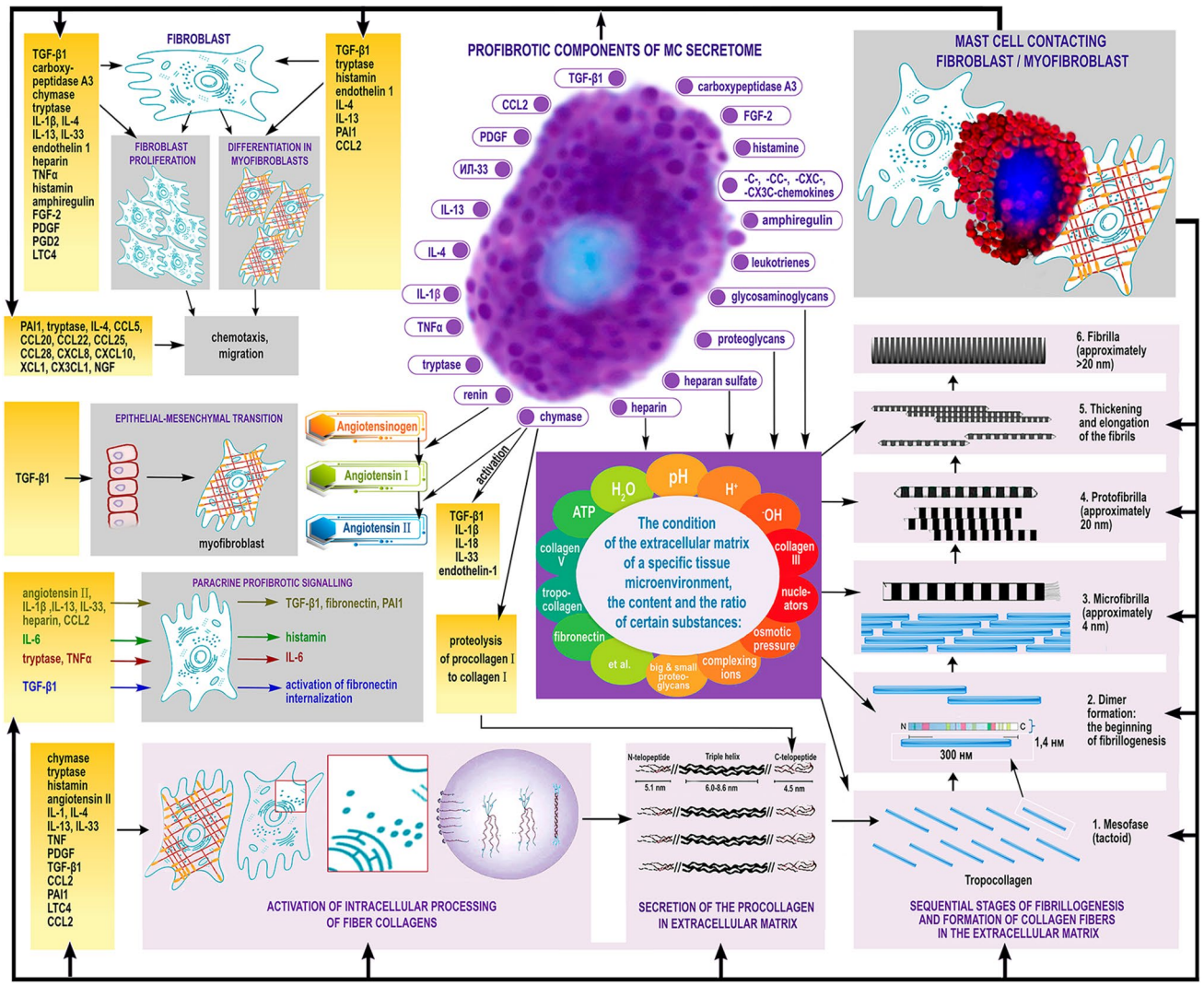

Fig. 6 Mast cell participation in the proteins biogenesis of the extracellular matrix fibrous component and the fibrillogenesis mechanisms. Mast cells are involved in both the formation of conditions for increased production of collagen proteins and the fibrillogenesis mechanisms. Various MC secretome components initiate an increase in fibroblast proliferation activity, their migration ability, transformation into myofibroblasts with alpha-smooth muscle actin accumulation and significant collagen synthesis activation, as well as the implementation of the epithelial–mesenchymal transition with the myofibroblasts additional formation. Thus, a pro-fibrotic phenotype of a specific tissue microenvironment is created. An equally important mechanism is the direct participation of MC in the collagen fiber formation, which occurs when contacting fibroblasts and being removed from them. In this case, the selective secretion of glycosaminoglycans and proteoglycans leads to the necessary parameters formation of an integrative metabolic buffer medium for inducing the fibrillogenesis start from collagen monomers and the sequential assembly of microfibril and protofibril fibers, ensuring fiber growth in length and thickness

assembly of fibrils begins in deep grooves or channels on the fibroblasts' surface (Birk and Trelstad 1986; Canty and Kadler 2005; Canty et al. 2004). Protofibrils with small diameters and uniform sizes and short lengths compared with mature fibrils accumulate in these microloci of the plasmalemma. Discoidin domain receptors 1 and 2, which are activated by contact with the ligand—collagen, are considered to be of great importance (Agarwal et al. 2019; Fridman and Agarwal 2019).

It is known that the polymerization of tropocollagen macromolecular complexes into microfibrils, fibrils, and fibers begins to occur autonomously under physiological conditions after the strictly defined molecular and chemical parameters of the intercellular matrix are reached. The electrostatic nature of the polypeptide chain interaction of contiguous macromolecules is essential in this process. Collagen molecules leaving the cytoplasm of fibroblast are about 300 nm in size and about 1.4 nm

thick, and they are located pericellularly forming molecular clusters—mesophases (Fig. 6). In these microloci of the intercellular matrix, the molecules are arranged parallel to each other, separated by water molecules forming the so-called tactoids or liquid crystals (Ghazanfari et al. 2016; Omel'yanenko 2009). Tropocollagen molecules are tens of nanometers removed from each other, preserving the mutual attraction determined by the balance between repulsion at short distances and attraction at long distances. Thus, collagen fibrillogenesis requires the formation of hydrogen-bond water clusters connecting recognition sites on opposite helices (Kuznetsova et al. 1998).

At this stage, intermolecular interactions are inherently labile, that is, hydrophilic–hydrophobic or electrostatic (Arques et al. 1996; Kuznetsova and Leikin 1999). The necessary molecules presence, including ATP, polyanions, cations, various types of collagen, etc., can change the balance of these labile interactions and, thus, contribute to a certain aggregation specific form for this tissue microenvironment (Fig. 6). An increase in pH enhances the hydrophobic interaction of collagen molecules, which initiates the formation of microfibrils without changing the molecular orientation (Ghazanfari et al. 2016).

Further approach of tropocollagen molecules, which occurs for several reasons, is necessary for the formation of supramolecular aggregates. First, the concentration of tropocollagen in the intercellular medium can increase significantly with an increase in the biosynthetic activity of fibroblast or other cells. In this case, obviously, the time factor is important for creating the necessary collagen protein concentration.

On the other hand, the concentration of glycosaminoglycans, which are able to absorb water and promote the convergence of tropocollagen molecules before polymerization, can increase in the environment of the tactoid location. In addition, a clear ratio of such parameters as pH, osmotic pressure, complexing ion concentration, and other components of the intercellular substance should be achieved (Ghazanfari et al. 2016; Harris and Lewis 2016; Omel'yanenko 2009). As a result, the adhesion forces arising between the molecules of tropocollagen result in supramolecular formations—microfibrils, which are filaments of 4–5 molecules of tropocollagen. Considering that MCs are practically the only source of heparin, as well as other glycosaminoglycans, in tissues, their close involvement in the mechanisms of fiber formation from collagen proteins becomes apparent (Fig. 6). The formation of collagen fibrils requires regulators, such as fibronectin and integrin collagen binding, as well as nucleators—collagen type V and XI (Kadler et al. 2008).

Collagens I, III, and V form mixed fibrils, which are present in all fibrous connective tissues, where type I is always the main type, while types III and V appear in different variable quantities depending on organ affiliation (Raspanti et al. 2018).

With the initiation of fibrillogenesis, each nucleation center competes with its neighbors for accessible molecules until all of them are exhausted. Since the extracellular matrix is the same for all the formed fibrils, they all have the same size without the intervention of any external factor. Relatedly, the aggregation of collagen molecules is very sensitive to the presence of external modulating factors, such as polyanions, ATP, or other molecules that do not even remain in the final product composition. The nature and operation of an unknown factor that causes the emergence of a particular architecture will be investigated in further research; and in this regard, mast cells continue to cause intriguing interest.

Thus, the adherence of MCs to fibroblasts may evidence the presence of certain potentials able to change the equilibrium state, in particular, result in the initiation of the fibrillogenesis (Figs. 5, 6). Localization of MCs near the fibers may evidence the participation of proteoglycans secreted by them in the elongation and thickening of fibrils (Figs. 1, 2, 3, 4). MC degranulation can be accompanied by the achievement of the required concentration of signal and structural molecules within a strictly limited microlocus. In addition, MC granules can act as nucleators—molecular loci initiating the start of collagen molecules polymerization and the fibrillogenesis process.

Glycosaminoglycans and fibrillogenesis

Mature fibrils are assembled by linear or lateral fusion of micro- and protofibrils (Trelstad 1982). Microfibrils are formed by combining 4–5 protofibrils adjacent to each other with lateral surfaces, their arrangement is achieved by alternating sections with a predominance of positive or negative charges. This regularity helps to increase the thickness of microfibrils (Cisneros et al. 2006). Selective aggregation of microfibrils into fibrils occurs due to a certain interaction with glycosaminoglycans (Cisneros et al. 2006; Graham et al. 2000; Ghazanfari et al. 2016; Omel'yanenko 2009). Collagen fibrils acquire a diameter of 15–500 nm depending on the type of tissue (Ricard-Blum 2011; Ricard-Blum and Ballut 2011).

As a result of the analysis of the electrostatic interaction between heparin and collagen, polymerization sites were shown and a molecular mechanism was proposed for the glycosaminoglycans effect on the morphology of collagen fibrils (Kulke et al. 2017). Heparin forms a bridge between the positive sites of two collagen triple helices and is able to adjust the distance between them, participating in determining the thickness of the fibril (Kulke et al. 2017). Considering the abundance of proteoglycans in the secretion of MCs (Ronnberg et al. 2012) and, primarily, heparin, it is possible to assume their active participation in the ordered

structuring of collagen fibrils. A variety of mechanisms for the secretome components selective secretion, including proteoglycans, allow MCs to participate in the molecular mechanisms forming spatial parameters of the fibrous component elements, including thickening, elongation, and histotopographic features that can be registered in microsections (Fig. 6).

There have been shown inducing heparin effects on the proliferation of dermal fibroblasts and their synthetic potency, in particular, the collagen protein biogenesis (Fan et al. 2008).

In vivo, the lateral growth of collagen fibrils is controlled by the binding of small leucine-rich proteoglycans (Kalamajski and Oldberg 2010). Special significance in determining the size and organization of collagen fibers is given to acidic glycosaminoglycans, in particular, keratan sulfate, chondroitin sulfate, dermatan sulfate, and hyaluronic acid (Borcherding et al. 1975).

Mast cells and dynamic tissue structuring

MCs accompanying and regulating the state of microcirculation vessels may indirectly participate in the dynamic structuring of the tissue (Fig. 3a) (Ghazanfari et al. 2016). The morphology of collagen fibrils is highly dependent on the medium pH: an increase in pH from approximately neutral (6.9–7.4)-to-slightly alkaline (7.6–8.1) led to an increase in the diameter of fibrils from 20 to 200 nm in the chicken cornea epithelium (Bard et al. 1993).

Proteoglycans with a large molecular mass are involved in the osmotic removal of water separating individual fibrils, whereas smaller ones are able to bind fibrils into a single complex fiber. Ultimately, the size of the fiber, and its ultrastructural and morphotopographic characteristics (thickness, the number of fibrils in its composition, shape) depends on the conditions of the tissue microenvironment, the biomechanical load on the organ tissues, the local homeostasis parameters, and integral collagen properties and glycosaminoglycan complexes.

In addition, the content of matrix metalloproteinases (MMPs) in MCs should be taken into account, since the degradation of collagen fibers depends on them. In this aspect, MCs, as a source of MMPs, appears to be an interesting object for research.

Histotopographic routing

It should be noted that the secretory activity of MCs is apparently capable of determining the newly formed collagen fiber localization. In particular, it should be said not only about fiber growth in thickness or length but also about the induction of fibrillogenesis in the necessary direction of the tissue microenvironment. This phenomenon of "histotopographic routing" can be determined by the MC localization in a strictly defined sequence in a limited volume of tissue. The synchronization mechanism of the secretory activity of multiple MCs in the tissue's volume and the forming fiber strictly ordered localization in accordance with morphogenetic conditions that remain insufficiently clear. As an assumption, it is possible to consider an algorithm for detecting the collagen fiber growth in length and the corresponding dosed secretion of MC components within strictly defined intercellular substance limits.

The MC secretory mechanism's unique regulation allows quickly and selectively delivering the required amount of biosynthetic activity products to the extracellular matrix in accordance with the trigger stimuli of the tissue microenvironment detected by the corresponding receptor apparatus (Redegeld et al. 2018; Robida et al. 2018). Moreover, MCs are able to adapt the size of secreted granules or exosomes in accordance with the activation of the type and degranulation synapses number and to fine-tune the secretion scale (Espinosa and Valitutti 2018). MC granules freely localized in an integrative-buffer metabolic medium and having autonomy of existence for some time may be considered as a morphological confirmation of this mechanism (Atiakshin et al. 2018, 2019). Part of the granules can come into contact with fibrous structures, the fact evidencing their participation in the remodeling process in the absence of the MC itself.

MC proteases in extracellular matrix remodeling

The expression of tryptases and chymases in MCs is dynamically changing depending on the existing tasks of remodeling intercellular substance (Atiakshin et al. 2018, 2019; Chen et al. 2017). Tryptase is capable of forming the profibrous phenotype of fibroblasts and their transformation into myofibroblasts using PAR2 signaling. Along with this, the ability of tryptase to stimulate the production of type I collagen fibronectin and laminin by fibroblasts and myofibroblasts should be noted. These effects are significantly potentiated by the ability of tryptase as a growth factor to activate the mitotic division of fibroblasts, increase migration activity, and cause an increase in their tissue number per unit volume. In addition, tryptase is able to change the ratio of MMP-1, -2/TIMP-1, -2 (Tan et al. 2018).

The chymase also has the ability to enhance the mitotic activity of fibroblasts, eliminating the contact inhibition of mitotic activity and contributing to the synthesis and secretion of type I and III collagen into the extracellular matrix, playing an important role in the development of fibrosis of the internal organs (Algermissen et al. 1999; Sakaguchi et al. 2002). Mast cell chymase promotes the proliferation of hypertrophic fibroblasts in the cicatricial tissue eliminating contact inhibition of mitotic activity and contributing to the type I and III collagen synthesis and secretion in the

extracellular matrix (Castagnoli et al. 1993; Chen et al. 2017).

Regarding fibrillogenesis, the direct ability of the chymase to enzymatically cleave a C-propeptide from type I collagen molecules attracts attention. Moreover, the same activity was detected in the chymase–heparin complex; the latter is secreted by the mast cell into the extracellular matrix in close connection with chymase (Kofford et al. 1997). The propeptides generated by the chymase can have feedback properties, affecting not only the processing of procollagen propeptides but also the regulation of feedback of procollagen biosynthesis.

The proteolytic activity of chymase is especially noteworthy, since it activates a number of target enzymes with profibrotic effects. For example, chymase is able to catalyze the formation of angiotensin II from angiotensin I more actively than the angiotensin-converting enzyme; angiotensin II, in turn, can provoke fibrous changes, i.e., by activating the biogenesis of collagen proteins (Sakaguchi et al. 2002; Sun et al. 1997). Chymase participation in the activation of transforming growth factor β (TGF-β) is of special significance.

TGF-β is an established regulator of extracellular matrix remodeling through transcriptional control of certain proteins in its composition. TGF-β1 can be produced in a specific tissue microenvironment not only by mast cells but also by others, including epithelial cells, fibroblasts, myofibroblasts, endothelial cells, platelets, macrophages, and neutrophils (Shimbori et al. 2019). Impaired TGF-β function leads to the progression of various diseases, including cancer, fibrosis, and other pathological conditions (Yun et al. 2019). The extracellular matrix serves for sequestration and formation of the increased TGF-β content in sites where it can be rapidly activated if necessary (Ramirez and Rifkin 2003). MC chymase can influence TGF-β activity. In turn, TGF-β can enhance the biogenesis of collagen fibers in several ways.

One of the mechanisms of this growth factor is associated with its ability to intensify the fibronectin internalization in fibroblasts and myofibroblasts, which represents a fast mechanism for the fibrillogenesis induction, independent of fibronectin transcription. Other mechanisms of fibrosis induction by TGF-β are associated with canonical, non-canonical, and endoglin–ALK signaling (Brown and O'Reilly 2019).

A recent study showed an increase in TGF-β1-positive mast cells in skin fibrosis (Di et al. 2016). Interestingly, the chymase and TGF-β1 can simultaneously release MCs; chymase, in this case, immediately activates latent complexes of TGF-β1 (Lindstedt et al. 2001; Zhao et al. 2008) and promotes the release of TGF-β1 into the extracellular matrix by human epithelial and endothelial cells (Taipale et al. 1995).

MC chymase supports the proliferation of hypertrophic fibroblasts in the cicatricial tissue eliminating contact inhibition of mitotic activity and contributing to the synthesis and secretion of type I and III collagen in the extracellular matrix (Castagnoli et al. 1993; Chen et al. 2017). TGF-β 1 induces epithelial–mesenchymal and endothelial–mesenchymal transition; this might be one of the principal mechanisms of morphogenesis, fibrosis, and cancer progression (Kalluri and Neilson 2003).

The chymase is able to activate endothelin-1. Extensive experimental data indicate that endothelin-1 may also have pro-fibrotic activity and take part in organ fibrosis (Jing et al. 2015; Shi-Wen et al. 2001; Xu et al. 2004). Elevated levels of endothelin-1 have been detected in some fibrotic diseases and in experimental pulmonary fibrosis (Park et al. 1997; Ross et al. 2010; Xu et al. 2004). Moreover, endothelin-1 may play a potential part in the generation of myofibroblasts (Kim and Chapman 2007; Widyantoro et al. 2010; Cipriani et al. 2015; Wermuth et al. 2016).

Conclusion

The results of the study performed provided evidence for the MCs close involvement in the fibrillogenesis process, which is especially noticeable in conditions of skin wound surface regeneration. Staining with metachromatic dyes and impregnation with silver appears to be an informative combination for studying the contribution of MCs to the fibril formation. Obviously, active MC participation in changing the state of the tissue microenvironment, namely, the secretion of glycosaminoglycans, contributes to the initiation of fibrillogenesis with further fiber formation in accordance with the conditions of dynamic structuring. The proposed combinations of histochemical protocols are informative for studying the regulatory mechanism of the collagen fiber formation in the extracellular matrix with MC participation. The histochemical method combination aimed at the simultaneous MCs' selective detection and fibrous elements of the intercellular matrix allows recognizing new details of the skin connective tissue remodeling mechanisms. The sufficient simplicity of MC identification opens wide prospects for researchers to study fibrillogenesis mechanisms in tissue microenvironment with cellular component participation. The results obtained can be used both for the assessment of adaptive processes and diagnosis of pathological conditions.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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