ORIGINAL RESEARCH

Manual Mobilization of Subcutaneous Fibrosis in Mice

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ABSTRACT

Objective: The aim of this study was to induce the remodeling of subcutaneous fibrosis in mice by the manual mobilization of skin and subcutaneous tissue.

Methods: Seven days after the induction of subcutaneous fibrosis, mice were divided into 3 groups: control, stretch, and manual mobilization. Stretch was achieved by elongating the trunk, and manual mobilization was achieved by using the indicator fingertip of both hands, side by side, touching the back and performing a brief stretch. Stretch or manual mobilization was performed once a day for 7 days.

Results: Fibrosis was present in the subcutaneous tissue of control animals, whereas brief stretch and manual mobilization were found to reduce fibrosis.

Conclusions: Mechanical stimulation through manual mobilization, or brief stretching, reduced subcutaneous fibrosis after tissue injury. (J Manipulative Physiol Ther 2018;41:359-362)

Key Indexing Terms: *Fibrosis; Extracellular Matrix; Musculoskeletal Manipulations; Mice*

INTRODUCTION

The wound healing mechanism after an injury is one of the most complex processes occurring in multicellular organisms. In mammals, the typical response to an injury is fibrotic scar formation, which re-establishes tissue integrity and function.[1] Adult human wounds heal with some degree of scar formation that may compromise function and appearance, and an estimated 230 million major surgical procedures are performed worldwide each year.[2,3] After skin injury, the mechanophysiological conditions drastically change during wound healing and considerably influence the degree of scarring.[4] The result can be a fine, thin scar that is barely perceptible or an exuberant fibrosis that can be dysfunctional and disfiguring.[5]

Extracellular matrix (ECM) component-accumulation, mainly collagen, and increased tissue stiffness are common features of fibrosis.[6] This accumulation alters the tissue mechanical properties, which, in turn, can deleteriously affect organ function.[7] Cells sense and respond to the ECM rigidity, which can regulate cell growth,[8] migration,[9] and differentiation.[10,11] Extracellular matrix rigidity also affects other parameters associated with fibrosis, including the deposition and organization of its own ECM.[12] It is now very clear that tissue stiffness may precede fibrosis or at least contribute to the ongoing fibrosis.

It is a common belief that once fibrosis has begun, it cannot be reversed. However, recent studies have illustrated that fibrosis can be reversed.[13] These studies suggest that alteration in the biomechanical properties of the ECM may be an important therapeutic target and that it is possible to modulate myofibroblast formation and fibrosis.[12] The aim of this study was to induce the remodeling of subcutaneous fibrosis in mice by the manual mobilization of skin and subcutaneous tissue.

METHODS

All procedures in this study were carried out in accordance with Brazilian legislation for experimentation with animals (n° 11.794, October 8, 2008). In addition, this study was approved by the Ethics Committee for the Use of Animals of the State University of Rio de Janeiro (0011/2012).

In Vivo Microinjury Study Design

Male Swiss mice, 12 weeks old, underwent a subcutaneous microsurgical injury on the subcutaneous tissue of

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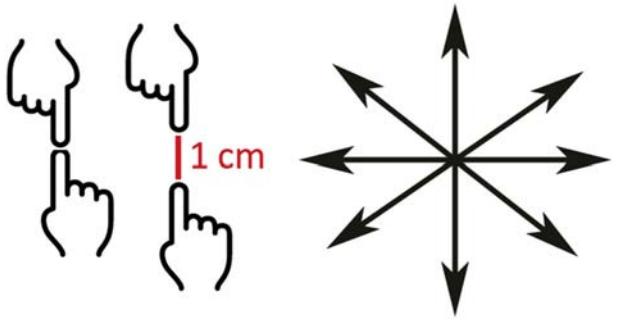

Fig 1. *Schematic representation of manual mobilization. Both fingertips are placed on animal's back with low pressure, and a brief stretch (1 cm) is applied for 10 seconds. This stretch is repeated in 4 directions, as represented by the arrows. Each treatment is composed of 3 cycles of stretch.*

the dorsal region, as previously described.[14] Seven days later, the animals were divided into 3 groups: the control group (n = 5), in which no treatment was performed; the stretch group (n = 5), in which the animals were stretched by elongating the trunk (20%-30% strain for 10 minutes, once a day for 7 days)[14]; and the manual mobilization group (n = 5), in which the backs of animals were manually mobilized for 3 cycles of 10 seconds, once a day for 7 days, using the pointer fingertips of both hands, side by side, to touch the back and perform a brief stretch of 1 cm in 4 directions (like an asterisk) (Fig 1).

Just before euthanasia, the skin adjacent to fibrosis was analyzed for pliability and mobility. For pliability testing, the skin was pinched with the thumb and the pointer fingertips. The following score was used, using a score system described by Dische: 0, normal tissue to no fibrosis; 1+, increase density; 2+, definitely increased density and firmness; and 3+, very marked density and fixation.[15] Because an important feature of mouse skin is its mobility,[16] a mobility test was created. For mobility testing, the skin was gently moved in a cephalo-caudal direction with a finger using soft pressure, (higher mobility = higher score). The following score was used: 3+, normal mobility; 2+, reduction of 20% to 40% in mobility; and 1+, reduction of >50% in mobility. The administrator of the test was blinded to the treatment groups.

Tissue Collection

Fourteen days after wounding, animals were euthanized by CO_2 exposure. In a region in the center of the animal's back where the fibrosis was induced, the skin, subcutaneous tissue, and muscular layer were collected, formalin fixed, and paraffin embedded. This way, the whole fragment contained fibrosis. Histologic sections (5 μm) were stained with hematoxylin and eosin and Mallory's trichrome. At least 3 sections were analyzed in each animal, with 10 sections discarded between them.

Results

The pliability test showed that the control group presented skin that was more attached to the subcutaneous tissue and muscular layer (3+) than the stretch (0+) and manual mobilization groups (0+). The mobilization test also demonstrated that the skin of the control group was more attached to the subcutaneous and muscular layers because the sliding was reduced in this group (1+) when compared to the stretch and manual mobilization groups (3+ for both).

In a pilot study, the formation of fibrosis was confirmed in the subcutaneous tissue 7 days after the wounding (data not shown). Normal mice have loose connective tissue in the subcutaneous layer (Fig 2a). Fourteen days after injury, there was a deposition of a fibrous and dense connective tissue (Fig 2b). Both stretching (Fig 2c) and manual mobilization (Fig 2d) were able to reverse the fibrosis in the subcutaneous tissue, and only loose connective tissue was observed.

Discussion

During wound healing or chronic pathologic conditions, such as fibrosis, tissue stiffness can increase over days to weeks due to the contractile activity of myofibroblasts.[17] This alteration warrants further research in the development of effective treatment options for fibrosis.

One important mechanism is mechanotransduction, which is a cell's ability to transform mechanical signs into biochemical responses.[1] It is known that when a mechanical force is applied to the ECM, it can influence the metabolic environment.[12,18] In our study, it is shown that a brief stretch applied to the scar tissue can reduce fibrosis and improve ECM organization. This finding addresses one of the most important problems of fibrosis, which is the increase and disorganization of the ECM. Our study corroborates previous findings of Bouffard et al,[14] who showed a decrease in collagen synthesis with stretching-type treatments. This effect is opposite to the results from the prolonged stretch and highlights the critical importance of "dose" (ie, duration, amplitude, frequency) in mechanically induced connective tissue remodeling. For example, a brief static stretch decreases proinflammatory cytokine TGFβ1 and collagen synthesis, but repetitive or high-amplitude mechanical input induces inflammatory stimuli and increases TGFβ1 and collagen synthesis.[14,19]

Identifying methods to modulate the mechanical properties of tissue microenvironment may lead to novel therapeutic approaches.[12] However, fibrosis was observed only in the subcutaneous tissue in our model, and this was a minor portion of the studied specimen that could be isolated, making it impossible to perform biochemical analysis. Hence, another model that allows deeper investigation into the mechanisms of how mechanical forces influence fibrosis remodeling needs to be developed. It is also important to evaluate the fibrosis. Here, we employed 2 methods to clinically evaluate fibrosis: the pliability test that has already been described[15] and validated

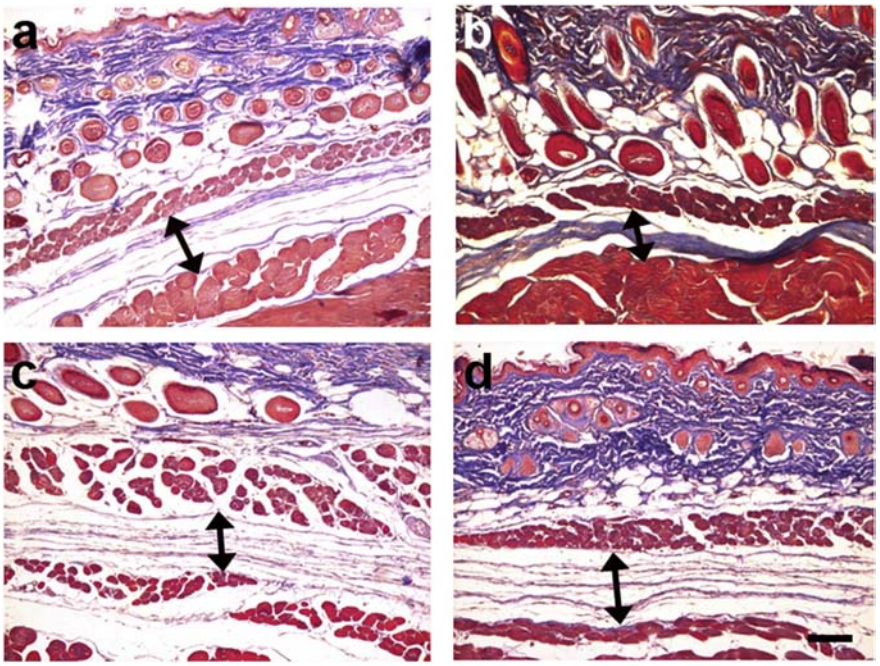

Fig 2. *Subcutaneous tissue in mice. In normal mice (a) not submitted to surgical procedures, the subcutaneous tissue (indicated by arrows) is composed of a loose connective tissue. In the control group (b), fibrosis is present and the subcutaneous tissue (indicated by arrows) is formed by dense connective tissue. In the stretch group (c) and the manual mobilization group (d), fibrosis is no longer observed, and the subcutaneous tissue (indicated by arrows) is composed of loose connective tissue. Mallory's trichrome. Bar – 100 μm.*

and the mobility test that we are proposing for the first time and which needs to be validated. In our study, the results of both tests were consistent and coherent with histologic results.

Moreover, findings from both studies (manual mobilization from the present study and the study by Bouffard et al with postural stretch[14]) suggest that stretch-induced ECM remodeling is an important natural mechanism limiting excessive scarring and fibrosis following injury.[14] Prevention and treatment of fibrosis and adhesion formation using stretch and mobilization are simple and cost-effective options for one of the most common problems after surgical procedures.

Limitations

This study is pioneering and still has some limitations that need to be addressed in the future. Among the points that need to be addressed are: (1) validation of the mobility test, (2) application of this technique (manual mobilization) to other models of cutaneous fibrosis, and (3) also to other animals and to humans. After all of the future confirmatory studies are performed, a new therapeutic approach will probably be available.

Conclusion

Mechanical stimulation through manual mobilization, or brief stretch, reduced subcutaneous fibrosis after tissue injury in a mouse model.

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Funding Sources and Potential Conflicts of Interest

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Contributorship Information

Concept development (provided idea for the research): M.A., A.M.-A.-C.
Design (planned the methods to generate the results): M.A., A.M.-A.-C.
Supervision (provided oversight, responsible for organization and implementation, writing of the manuscript): A.M.-A.-C.
Data collection/processing (responsible for experiments, patient management, organization, or reporting data): M.A., A.M.-A.-C.
Analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): M.A., A.M.-A.-C.

Literature search (performed the literature search): M.A., A.M.-A.-C.

Writing (responsible for writing a substantive part of the manuscript): A.M.-A.-C.

Critical review (revised manuscript for intellectual content, this does not relate to spelling and grammar checking): M.A., A.M.-A.-C.

Practical Applications

- This study found that fibrosis can be remodeled, and mechanical stimulation can reduce subcutaneous fibrosis in mice.
- Specifically, subcutaneous fibrosis can be reduced through manual mobilization in mice.

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