



# From stem cells to myofibroblasts: modulating palatal wound healing



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## Introduction

Healing of open wounds involves wound contraction and scar formation. These generally beneficial processes are a main cause of functional and/or growth impairment after cleft palate repair (fig. 1). This often requires additional orthodontic or surgical treatment. Our hypothesis is that stem cells from the blood enter wound sites and differentiate into fibroblasts. Subsequently, they differentiate into myofibroblasts, which mediate wound contraction and subsequent scarring (fig. 2). In this study we show that stem cells from the blood are contributing to palatal wound healing.



Figure 1. Scar formation after cleft palate surgery impairs the development of the upper jaw.

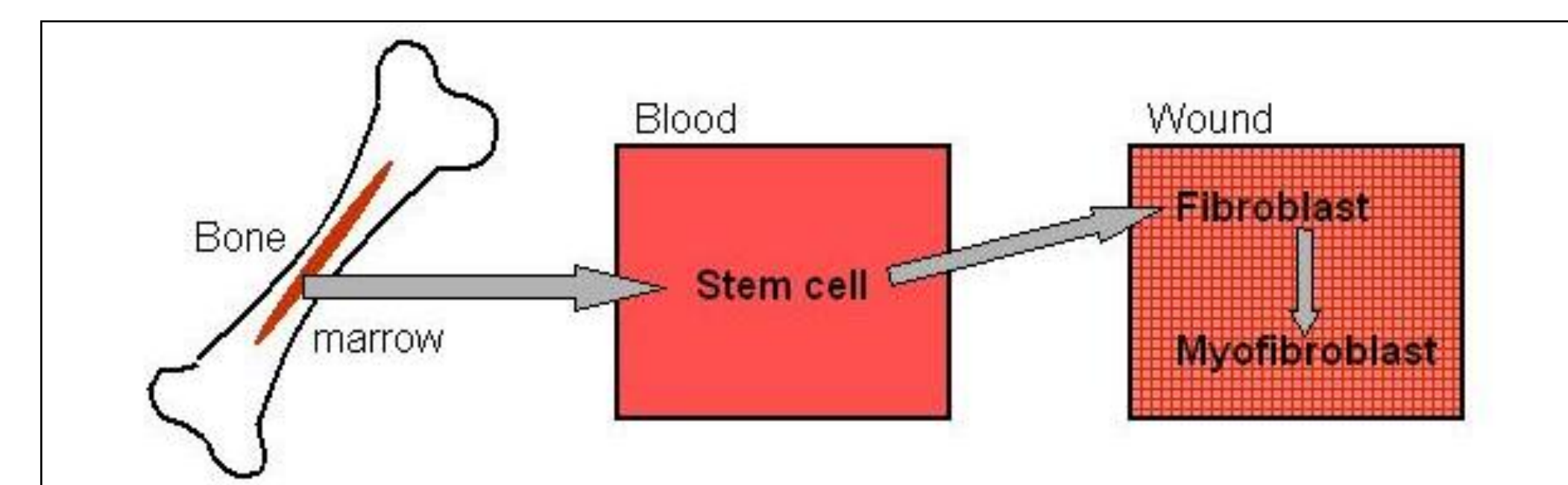


Figure 2. Our hypothesis: Stem cells from the bone marrow enter wound sites and differentiate into myofibroblasts.

## Material and Methods

- Animals: Female wild-type rats and GFP transgenic male rats (fig. 3)
- The females were exposed to 10 Gray irradiation
- Bone marrow cells were collected from GFP male femora
- Bone marrow cells were transplanted to the syngenic female rats

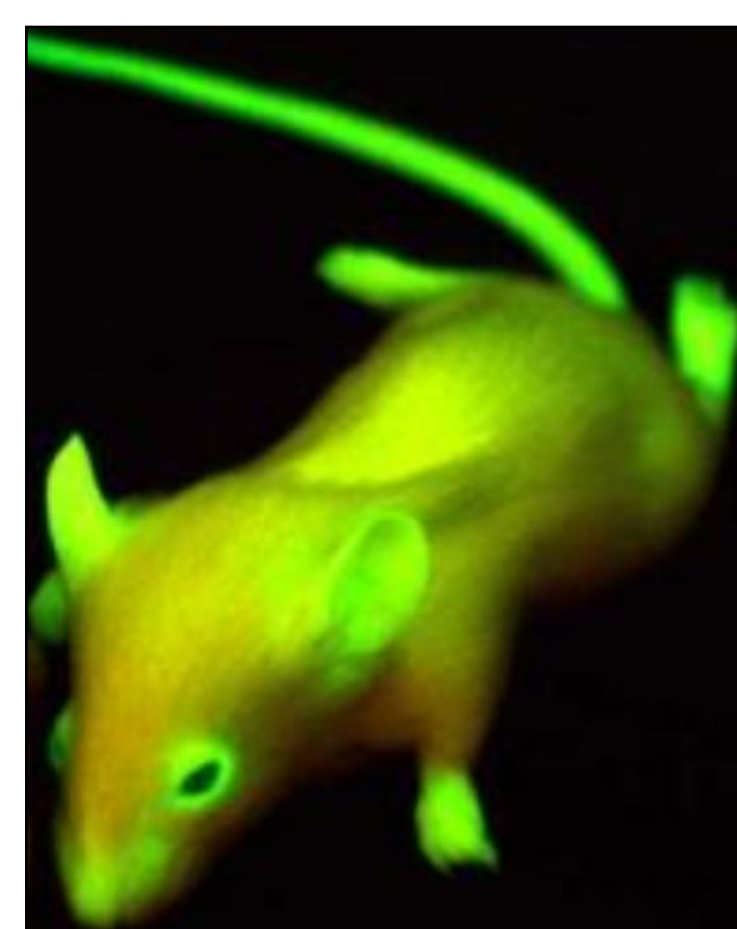


Figure 3. GFP transgenic animals appear green under UV-light.

- Five weeks after the BMT, blood was analyzed by FACS
- Seven weeks after the BMT, a standardized palatal wound was made (fig. 4)
- Nine weeks after the BMT, tissues were collected
- Detection: Alpha smooth muscle actin and green fluorescent protein by antibodies

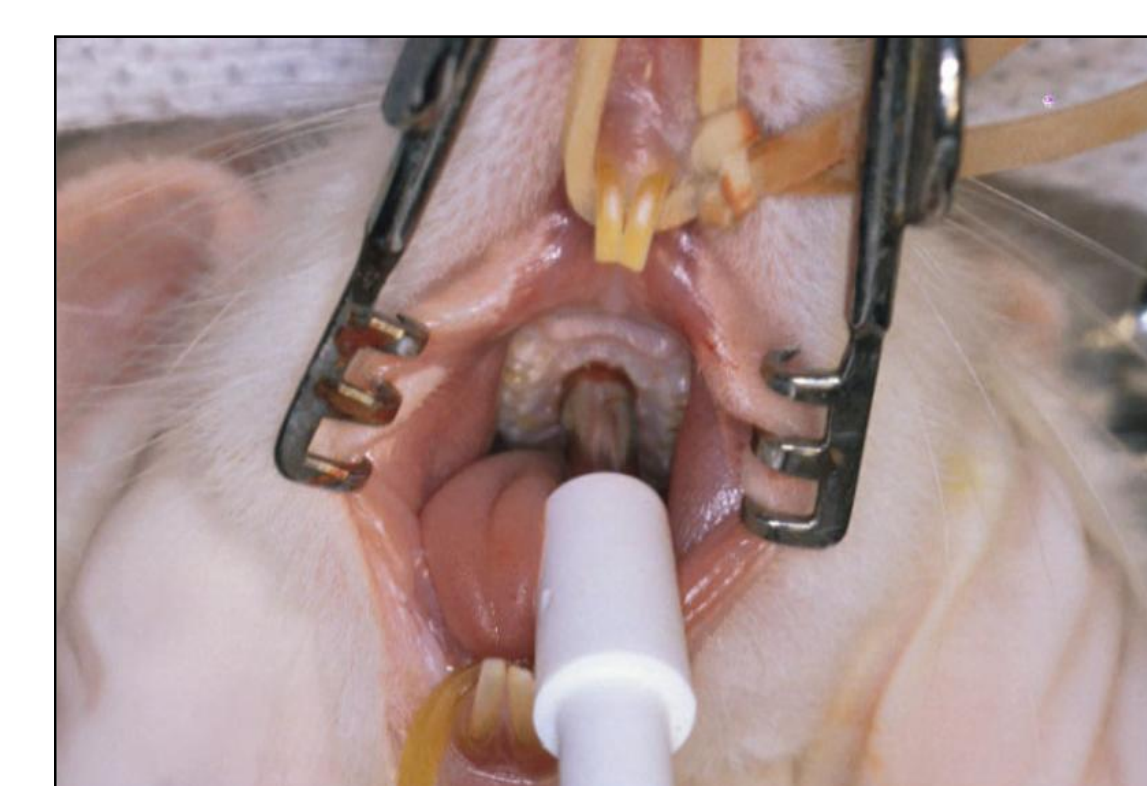


Figure 4. A standardized wound is made in the rat palate with a biopsy punch.

## Results

Figure 5 shows a histogram of the FACS results. Figure 5A are GFP positive lymphoid-derived cells. The GFP positive myeloid-derived cells are represented in figure 5B. These regions are 86% and 94% (average standard deviation) respectively. Figure 6 shows the fluorescence of wounded and control palatal cryosections. Figure 7 shows a double staining for alpha smooth muscle actin and green fluorescent protein.

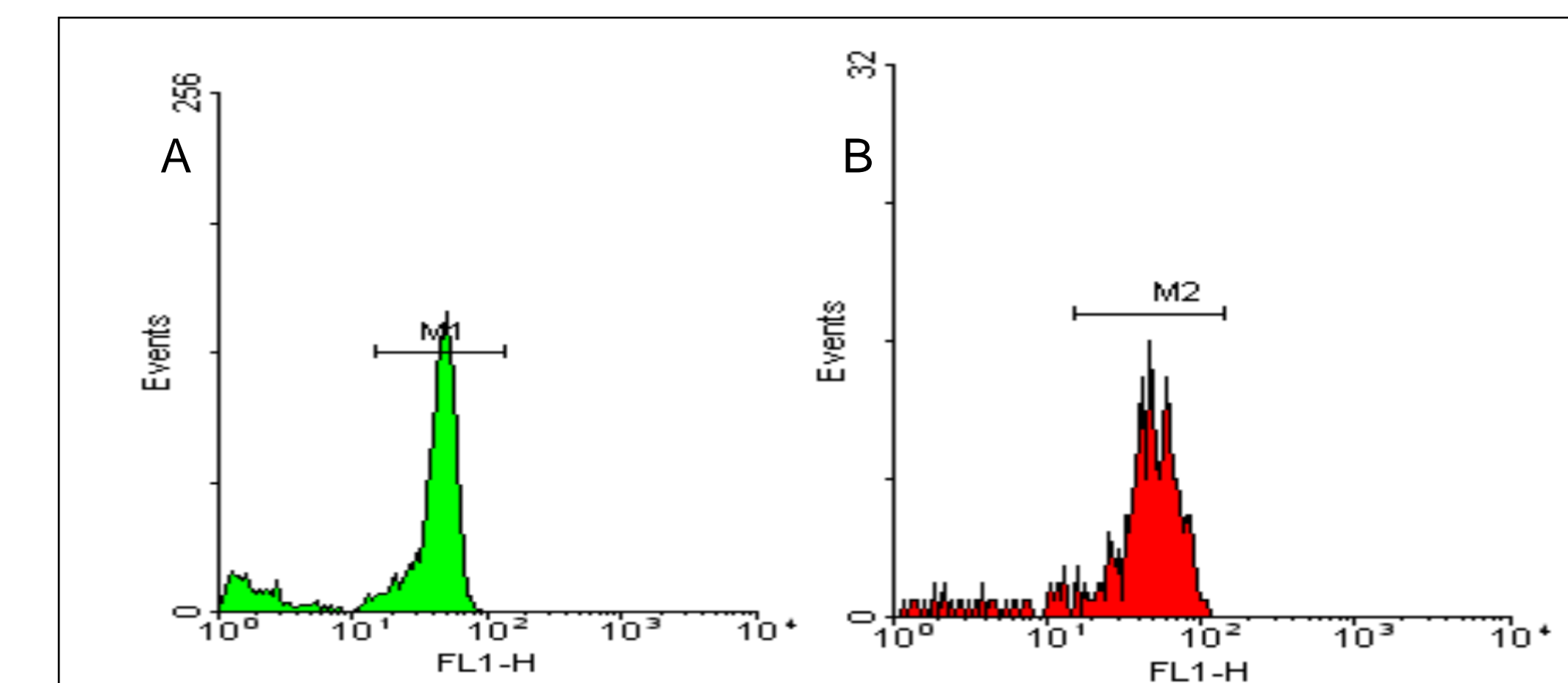


Figure 5. These FACS histograms represent the lymphoid (A) and myeloid (B) cells, which are 86% and 94% GFP positive, respectively.

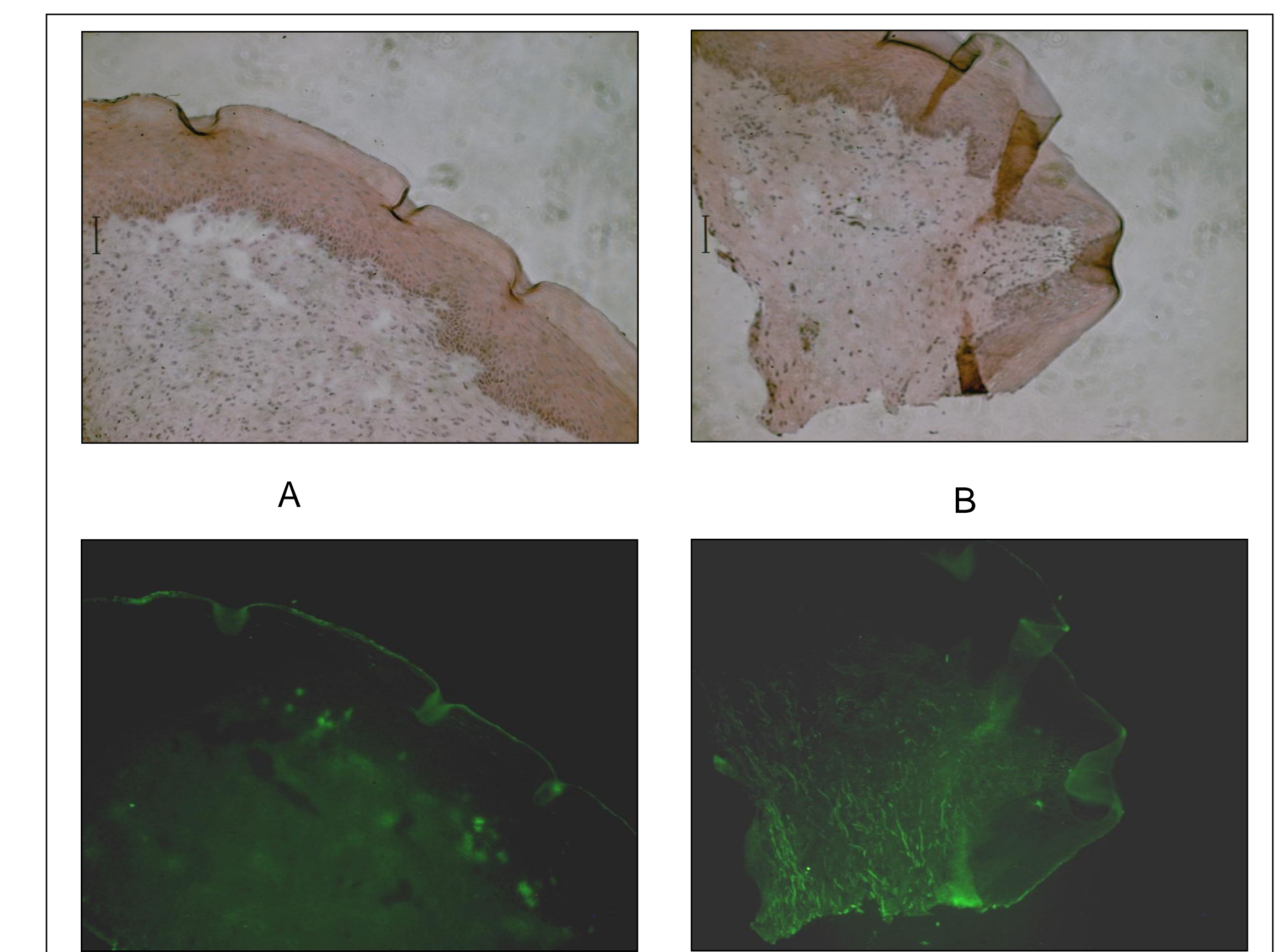


Figure 6. Wound tissue (A) and control tissue (B) 20x, before (lower panel) and after (upper panel) HE staining. The wound tissue shows a higher number of cells, and newly formed collagen compared to the control tissue (A). Fluorescence of GFP in the control tissue is almost absent under UV-light, whereas the wound tissue contains GFP positive cells. The autofluorescence of "old" collagen is only visible in the control section (B).

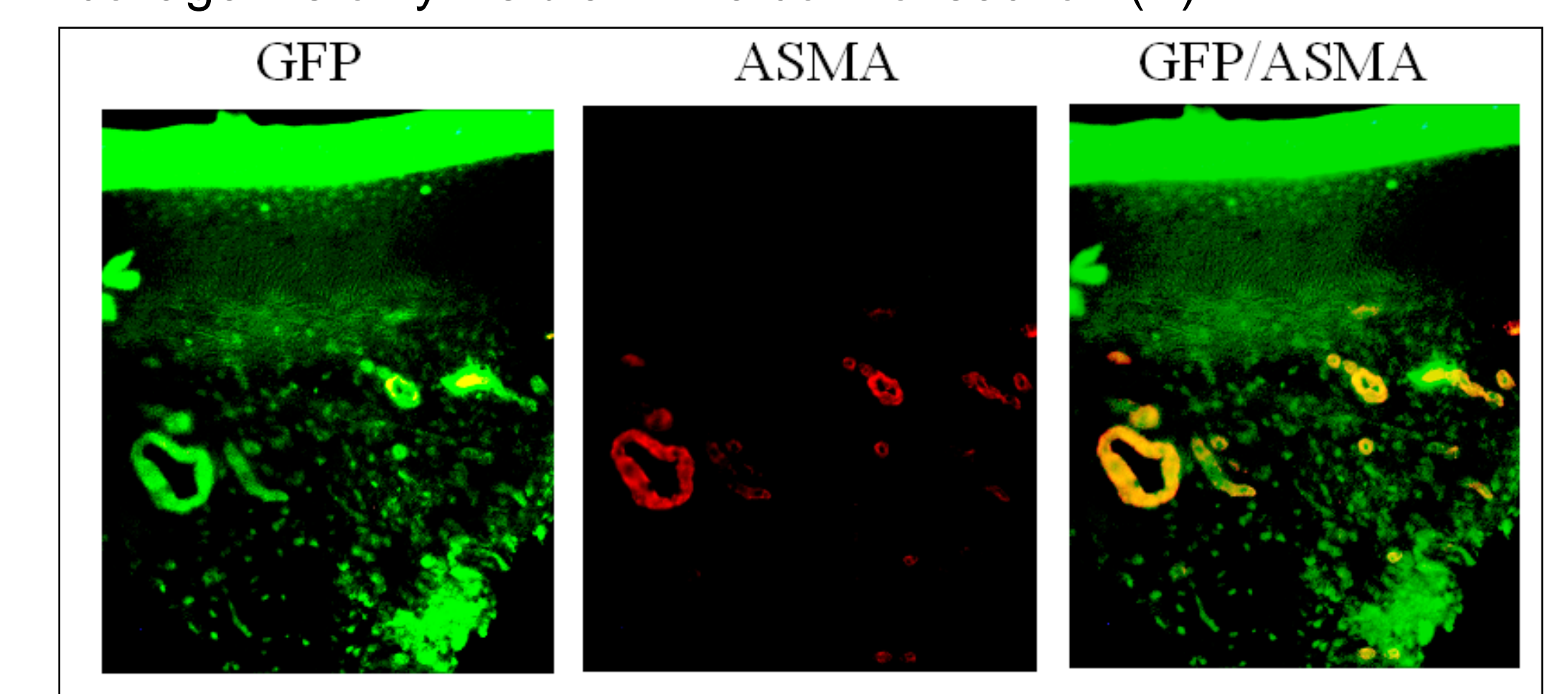


Figure 7. GFP+ rat palate, stained with a GFP fluorescent antibody (green) and alpha smooth muscle actin (red).

## Conclusions

Our preliminary results show that:

- Bone marrow transplantation in rats is a successful technique
- Blood cells are GFP positive after a GFP bone marrow transplantation
- GFP positive cells can be monitored
- Alpha smooth muscle actin can be visualized

These results also suggest that stem cells from the blood enter wound sites and contribute to wound healing.

Currently, we are characterizing cell types in the wound tissue. In the future, we want to modulate stem cell differentiation to myofibroblasts.