

3D-printed chitosan scaffolds for the improved in-vivo wound healing in diabetic rats

In the tissue engineering context, fabrication of porous three dimensional (3D) printed chitosan (CH) scaffolds for skin tissue regeneration and their behavior in terms of biocompatibility, cytocompatibility and toxicity toward human fibroblast (Nhdf) and keratinocyte (HaCaT), was discussed. These scaffolds, referring to a grid with filament being 200 μm distant from each other, were characterized by an accurate geometry and good surface homogeneity in terms of pore size and distribution: on the surface of the filaments the pores (Feret diameter: $3.5 \pm 3 \mu\text{m}$) presented a preferential orientation, whereas regular interconnected and layered pore structure within the filaments. After 35 days the total number of metabolically active cells in to the 3D environments was comparable or higher with respect to the corresponding value to 2D at the same time point.

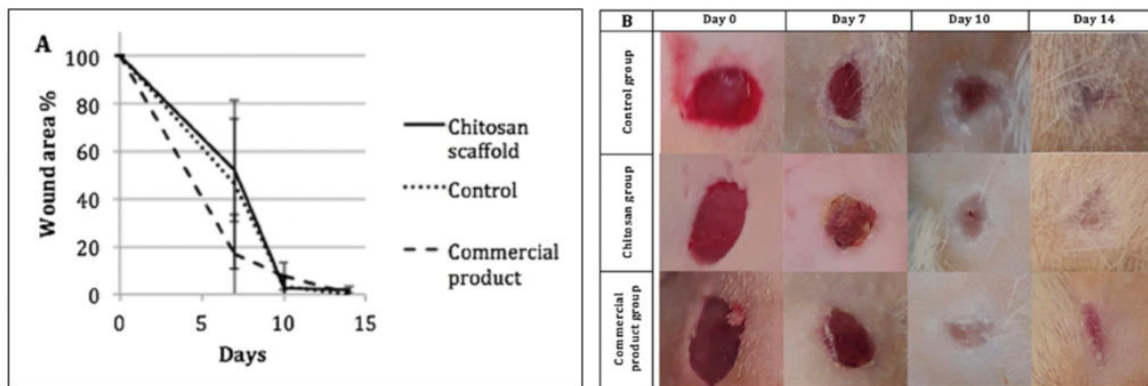


Fig. 1. (a) contraction of wounds expressed as percent of initial area 7, 10 or 14 days after wound infliction; (b) representative images of wound healing in treated and untreated groups: each photo corresponds to an area of 1 cm^2 .

The best cell growth was obtained after 35 days on 3D scaffold when the Nhdf and HaCaT cells, seeded together, filled the pores in the scaffolds as measured by neutral red staining and confirmed by scanning electron microphotographs. An early skin-like layer consisting of a mass of fibroblast and keratinocyte cells growing together was observed. The tests of 3D printed scaffolds in wound healing carried out on streptozotocin-induced diabetic rats demonstrate that 3D printed scaffolds improve the quality of the restored tissue with respect to both commercial patch and spontaneous healing. As shown in Figure 1, starting from round excisions having an area of about 0,3 cm^2 , seven days later, in animals treated with chitosan scaffolds or untreated ones, wounds appeared reduced of about 50 % with respect to initial area (chitosan scaffold: $52 \pm 21\%$); control group ($46 \pm 35\%$), while a more consistent, although not significant ($p > 0.05$) contraction in wounds treated with the commercial product was observed (reduction to about $20 \pm 17\%$). After 10 days, healing could be considered completed in animals treated with chitosan scaffolds or commercial product, while in control animals scabs were still present. After 14 days, all wounds were completely healed and only scars were visible. Representative images of wounds at different time points are collected in Figure 1b. On the whole, visual inspection of wounds confirmed that treatment with chitosan scaffolds improved and accelerated wound healing with respect to untreated animals, but did not show significant differences with respect to the use of a commercial product. Differences in the quality of wound healing was appreciated by histological

analysis. Figure 2 shows the comparison of a wound treated with chitosan hydrogel with respect to spontaneous healing and a commercial product, 7 days after wound infliction. The scarlet area on the side of both images marks the limit of original wound, evidencing collagen structure.

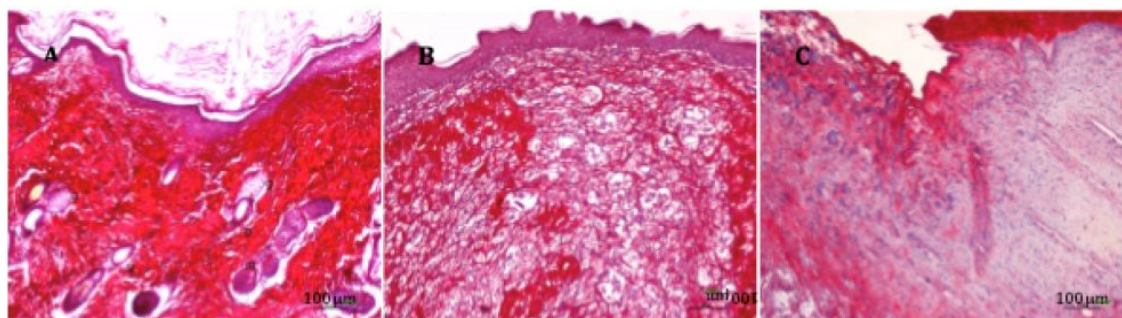


Fig. 2. Histological staining Hematoxylin + picro-sirius red of tissue excised from wounds treated with chitosan hydrogels (A) or spontaneously healed (B) for 7 days or a commercial product (c) for 14 days.

In Figure on the left, the intense purplish red area is indicative of a more intense tissue reorganization, the tissue is completely reorganized and the epidermis repaired (purple layer up), with a huge amount of collagen (scarlet red) that is not coarse anymore but rather mature and organized into fibrils, with respect to the pale pink area of the photo on the center and the right, where tissue organization and collagen is very scarce. Epidermis (purple layer up) is present only in the wound treated with chitosan hydrogel. On the left, it is not possible anymore to distinguish the site of wound infliction, since. Moreover, tissue annexes are present such as blood vessels (v), sebaceous glands (g), hair follicles (h) and erector pili muscles (p). On the other hand, wounds treated with the commercial product showed a non-ordered distribution of loose collagen fibers surrounding an area of tissue, that appears like fat tissue, with a functionality that is still compromised and without evidence of tissue annexes.

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Publication

[3D-printed chitosan-based scaffolds: An in vitro study of human skin cell growth and an in-vivo wound healing evaluation in experimental diabetes in rats.](#)

Intini C, Elviri L, Cabral J, Mros S, Bergonzi C, Bianchera A, Flammini L, Govoni P, Barocelli E, Bettini R, McConnell M

Carbohydr Polym. 2018 Nov 1