Introduction
Normal collagen hydrogels (NCH), developed by Bell and co-workers, associated with sheets of keratinocytes are already used as skin temporary coverage (Apligraf®). Nevertheless, NCH have several drawbacks such as extensive collagen hydrogel contraction, inhibition of cell growth and poor mechanical properties. In addition, cell death by apoptosis is observed. Because of their characteristics, NCH cannot be used as permanent grafts. In a previous study, we enhanced the properties of hydrogels by increasing the collagen concentration up to 3 mg/ml (CCH3). We observed a significantly lower contraction and better cell growth [1]. In the present study, we developed a concentrated-collagen-based hydrogel at 5 mg/ml (CCH5) to improve collagen hydrogel properties. In order to assess its capability to promote tissue repair, fibroblast phenotype within CCH5 was investigated.

Materials and Methods
Collagen hydrogels concentrated at 5 mg/ml (CCH5) were performed by mixing at room temperature 10 mg/ml collagen solution with culture media, sodium hydroxide 0.1 M and suspension of dermal fibroblasts (75000 cells/hydrogel). NCHs at 0.66 mg/ml were used as control. The effect of collagen concentration on hydrogel contraction and cell growth was evaluated for 21 days of culture. Remodelling activities (MMP2, MMP1 and MT1-MMP) were assessed by zymography or western blotting, cytokine expression (KGF and VEGF) detected by RT-PCR. In addition, the number of apoptotic cells was assessed by TUNEL test.

Results

Increasing initial collagen concentration within hydrogels significantly inhibited gel contraction. Contrary to NCH, CCH5 contraction was delayed until day 7. At day 21, the hydrogel area was 33 times bigger than NHC area (18 times for CCH3) (Fig. 1). Compared to NCH and CCH3, cell growth was improved with the CCH5 scaffold (10 and 1.5 times respectively). As fibroblasts secrete growth factors that favour wound healing, CCH5 could be an interesting cell carrier to promote tissue repair. The phenotypic study of fibroblasts revealed a sustained VEGF expression in CCH5 over the 21 days of culture. In contrast, VEGF transcripts were not detected within NCH from day 14 (Fig. 2). Hence, concentrated collagen hydrogels have more abilities to favour neovascularization than normal hydrogels. KGF, secreted by fibroblasts, promotes epidermis development. The results obtained by Real Time RT-PCR did not show significant differences between both types of hydrogels. Nevertheless, the amount of KGF available for keratinocytes would be more important due to the better cell growth in CCH5. The matrix breakdown was increased within NCH. Indeed, MMP2 and MMP1 production was higher than in CCH5 and MT1-MMP expression was detected at each culture time point (Fig. 2). TUNEL test evidenced that concentrated collagen hydrogels protected fibroblasts from apoptosis as few apoptotic cells were observed (Table 1).

Table 1. Percentage of apoptotic cells.

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<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tr>
<td>NCH</td>
<td>8.6 ± 7.5</td>
<td>44 ± 7.7</td>
<td>56 ± 5.6</td>
</tr>
<tr>
<td>CCH5</td>
<td>0</td>
<td>0</td>
<td>8 ± 2</td>
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Discussion and Conclusions
We demonstrate concentrated collagen hydrogel at 5 mg/ml can be a good candidate for dermal substitution. CCH5 hydrogels are stable, increase fibroblast cell growth and can promote neovascularization and epidermic development.

References

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Disclosures
The authors have nothing to disclose.