

SUPPLEMENTARY MATERIAL

Table S1. Levels of phospholipid fatty acids in epidermis and dermis of skin substitutes after EFA supplementation.

| Fatty acids | Epidermis | | | | | | Dermis | | | | | |
|--|--------------------------------------|----------------------------|---------|--------------------------------------|---------------------------|---------|--------------------------------------|----------------------------|---------|--------------------------------------|---------------------------|---------|
| | Mean \pm SD (μ g/g of tissue) | | P-value | Mean \pm SD (μ g/g of tissue) | | P-value | Mean \pm SD (μ g/g of tissue) | | P-value | Mean \pm SD (μ g/g of tissue) | | P-value |
| | Substitute ⁻ | Substitute ^{ALA+} | | Substitute ⁻ | Substitute ^{LA+} | | Substitute ⁻ | Substitute ^{ALA+} | | Substitute ⁻ | Substitute ^{LA+} | |
| Saturated FAs | | | | | | | | | | | | |
| 14:0 (Myristic acid) | 35.1 \pm 12.7 | 35.6 \pm 15.0 | NS | 28.7 \pm 7.0 | 39.0 \pm 3.8 | NS | 70.7 \pm 12.0 | 56.8 \pm 26.2 | NS | 71.4 \pm 30.1 | 44.2 \pm 12.1 | NS |
| 16:0 (Palmitic acid) | 282.3 \pm 133.5 | 271.3 \pm 87.7 | NS | 186.3 \pm 25.9 | 240.4 \pm 24.4 | <0.0001 | 449.3 \pm 63.6 | 354.8 \pm 141.4 | NS | 453.1 \pm 139.2 | 334.4 \pm 81.0 | NS |
| 18:0 (Stearic acid) | 220.8 \pm 105.2 | 226.9 \pm 71.1 | NS | 151.4 \pm 19.9 | 198.0 \pm 27.8 | <0.0001 | 424.3 \pm 87.1 | 381.2 \pm 142.5 | NS | 401.2 \pm 110.6 | 335.1 \pm 55.2 | NS |
| n-3 PUFAs | | | | | | | | | | | | |
| 18:3n-3 (ALA) | 0 \pm 0 | 3.5 \pm 5.9 | NS | 0 \pm 0 | 0 \pm 0 | NS | 0 \pm 0 | 22.5 \pm 14.0 | NS | 0.6 \pm 1.4 | 0 \pm 0 | NS |
| 20:5n-3 (EPA) | 2.5 \pm 2.8 | 30.3 \pm 13.5 | <0.0001 | 0.38 \pm 0.86 | 1.8 \pm 1.4 | NS | 0 \pm 0 | 55.6 \pm 31.0 | <0.0001 | 3.9 \pm 4.9 | 0 \pm 0 | NS |
| 22:5n-3 (DPA) | 9.2 \pm 7.2 | 34.5 \pm 11.7 | <0.0001 | 6.7 \pm 1.2 | 7.5 \pm 0.7 | NS | 42.3 \pm 11.8 | 123.9 \pm 34.2 | <0.0001 | 32.3 \pm 9.8 | 23.1 \pm 6.0 | NS |
| 22:6n-3 (DHA) | 10.3 \pm 7.8 | 13.9 \pm 4.8 | NS | 7.8 \pm 1.8 | 8.4 \pm 1.8 | NS | 50.6 \pm 21.1 | 42.0 \pm 9.4 | NS | 40.7 \pm 16.1 | 28.7 \pm 6.9 | 0.011 |
| n-6 PUFAs | | | | | | | | | | | | |
| 18:2n-6 (LA) | 116.3 \pm 74.0 | 114.8 \pm 65.7 | NS | 69.7 \pm 23.3 | 105.7 \pm 12.8 | <0.0001 | 100.8 \pm 40.6 | 152.5 \pm 77.01 | NS | 131.4 \pm 37.4 | 200.2 \pm 68.8 | NS |
| 18:3n-6 (γ -linolenic acid) | 0 \pm 0 | 0 \pm 0 | NS | 1.5 \pm 3.4 | 1.6 \pm 3.9 | NS | 1.1 \pm 1.8 | 0.5 \pm 0.77 | NS | 0.6 \pm 1.5 | 1.6 \pm 1.8 | NS |
| 20:3n-6 (dihomo- γ -linolenic acid) | 15.2 \pm 11.9 | 12.5 \pm 6.7 | NS | 12.2 \pm 2.6 | 18.4 \pm 3.6 | NS | 15.5 \pm 2.1 | 16.8 \pm 7.65 | NS | 20.4 \pm 6.6 | 16.7 \pm 6.2 | NS |
| 20:4n-6 (AA) | 50.8 \pm 41.7 | 39.0 \pm 13.6 | NS | 32.5 \pm 6.5 | 51.5 \pm 8.4 | 0.0031 | 418.7 \pm 111.1 | 251.2 \pm 87.7 | <0.0001 | 348.1 \pm 175.2 | 334.7 \pm 79.4 | NS |
| n-7 MUFAs | | | | | | | | | | | | |
| 16:1n-7 (Palmitoleic acid) | 157.2 \pm 87.8 | 163.1 \pm 95.8 | NS | 120.9 \pm 31.6 | 158.0 \pm 19.8 | NS | 66.7 \pm 3.9 | 60.9 \pm 27.9 | NS | 131.1 \pm 135.1 | 42.9 \pm 10.9 | NS |
| 18:1n-7 (Vaccenic acid) | 252.1 \pm 132.0 | 199.5 \pm 66.9 | NS | 191.7 \pm 30.0 | 226.3 \pm 31.5 | NS | 214.4 \pm 58.5 | 120.3 \pm 44.3 | 0.0232 | 266.1 \pm 171.6 | 108.2 \pm 20.8 | 0.043 |
| n-9 MUFA | | | | | | | | | | | | |
| 18:1n-9 (Oleic acid) | 594.8 \pm 347.7 | 561.1 \pm 252.4 | NS | 398.2 \pm 71.7 | 491.7 \pm 53.2 | <0.0001 | 492.8 \pm 102.6 | 381.3 \pm 135.9 | NS | 601.2 \pm 315.7 | 300.6 \pm 55.8 | 0.0002 |
| Totals | | | | | | | | | | | | |
| Total PUFAs | 219.0 \pm 152.8 | 259.7 \pm 121.9 | NS | 147.2 \pm 46.4 | 220.1 \pm 25.3 | NS | 796.0 \pm 195.1 | 728.9 \pm 271.0 | NS | 731.0 \pm 223.1 | 750.6 \pm 183.0 | NS |
| Total MUFAs | 1054.3 \pm 580.7 | 955.8 \pm 416.2 | NS | 741.4 \pm 132.7 | 911.9 \pm 106.9 | NS | 892.0 \pm 187.9 | 625.8 \pm 219.1 | NS | 1093.4 \pm 617.0 | 519.7 \pm 80.5 | 0.01 |
| Total FAs | 1913.7 \pm 1021 | 1895.9 \pm 764.9 | NS | 1347.0 \pm 230.3 | 1719.0 \pm 160.1 | 0.0116 | 2813.2 \pm 604.4 | 2232.2 \pm 825.4 | NS | 2818.0 \pm 890.8 | 2088.5 \pm 439.6 | NS |

Abbreviations : AA, arachidonic acid; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acid; SD, standard deviation.

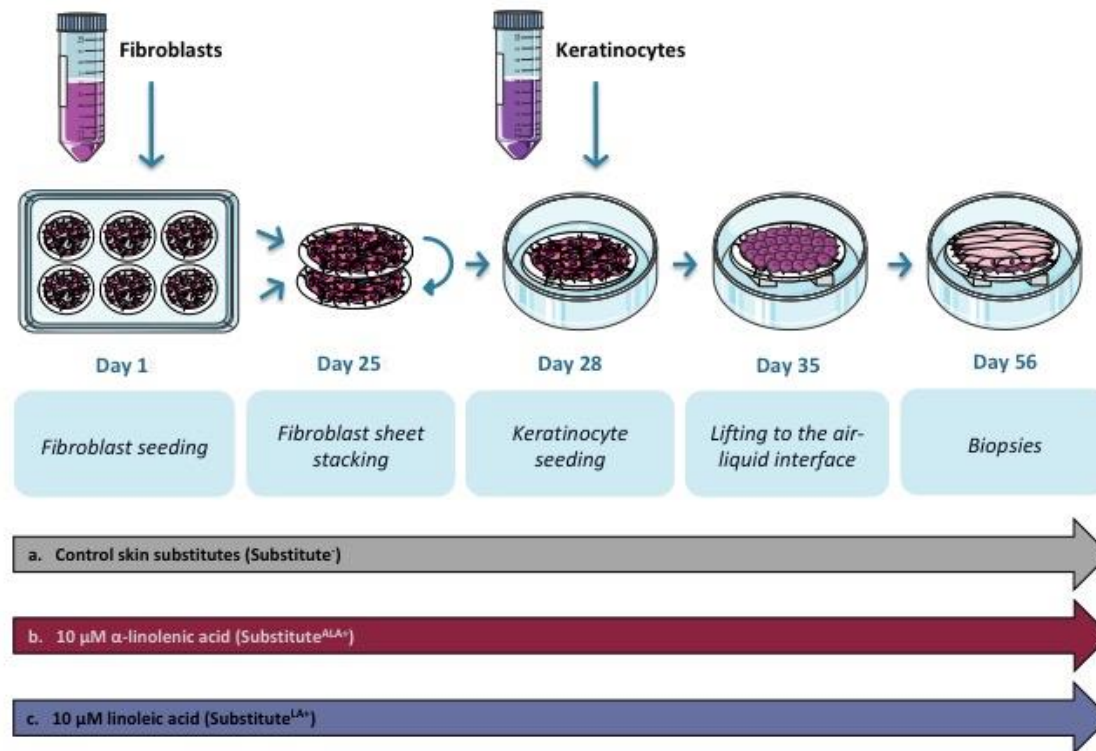


Figure S1. Schematic overview of skin substitute reconstruction by the self-assembly method. (Day 1) Fibroblasts were cultured in 6-well plates to form manipulatable sheets. (Day 25) Two sheets were superimposed in a 100 mm Petri dish and incubated for three days to form the dermal layer. (Day 28) Keratinocytes were seeded on the dermal equivalent to form a new epidermal layer and after seven days of culture (Day 35) the substitutes were raised to the air-liquid interface for 21 days. After a total of 56 days of culture, biopsies of the skin substitutes were taken and analyzed.

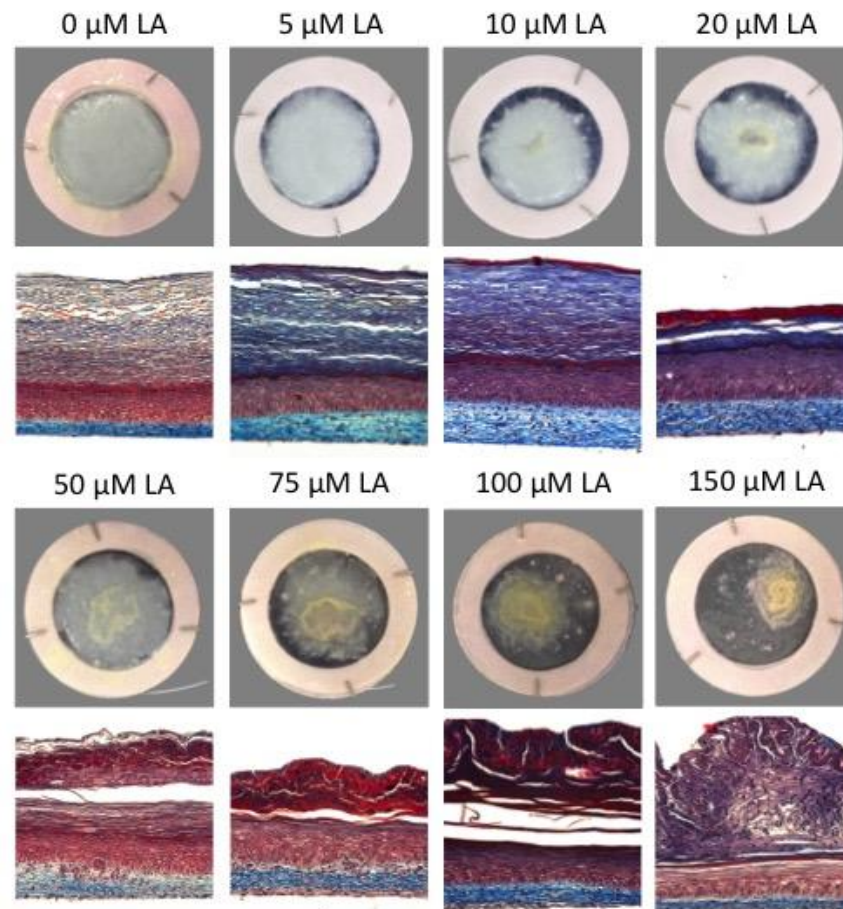


Figure S2. Effects of LA on the morphology of the skin substitutes produced using a range of concentrations (5 μM, 10 μM, 20 μM, 50 μM, 75 μM, 100 μM and 150 μM).

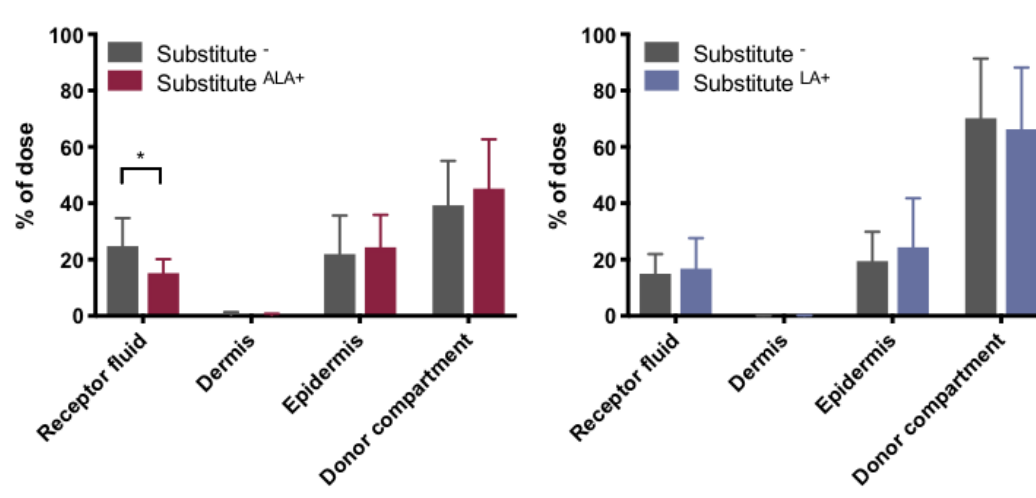


Figure S3. *In vitro* percutaneous absorption of testosterone through skin substitutes, expressed as percentage of the dose recovered in the different compartments after 24 h. Values are mean \pm SD (3 donors, 6 skin substitutes per donor), Two-way ANOVA followed by Tukey's *post-hoc* test. * $P < 0.05$.

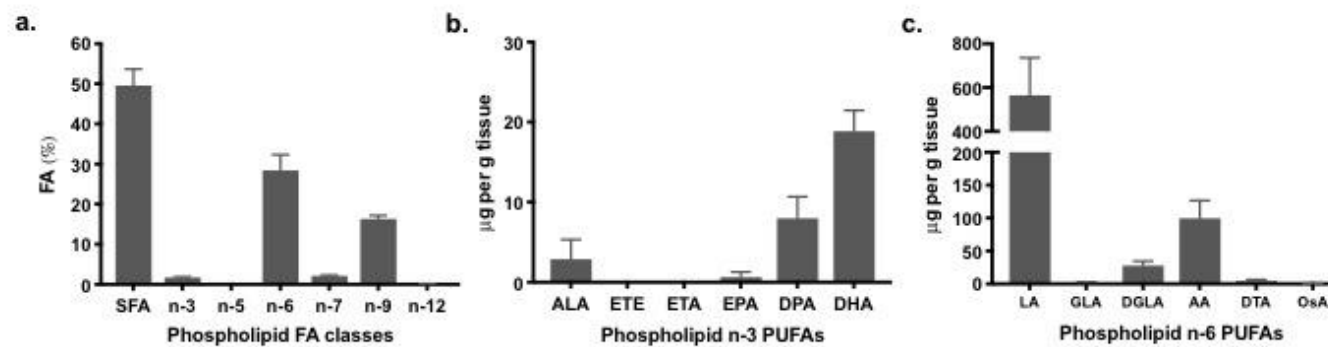


Figure S4. Human skin lipid profile. (a) Proportion of different FA classes in phospholipids of human epidermis. (b) n-3 PUFAs and (c) n-6 PUFAs in phospholipids of human epidermis. Values are means \pm SD ($n=6$). Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; ETE, eicosatrienoic acid; GLA, γ -linolenic acid; LA, linoleic acid; OsA, osbond acid; PUFAs, polyunsaturated fatty acids.