

Scaffold production

The scaffold was produced as described in [<https://doi.org/10.1016/j.carbpol.2022.119126>].

Briefly, 20ml of a 4% chitosan solution prepared in 2.5 v/v% glacial acetic acid/distilled water containing nHA rods (70% w/w) was mixed with 4mL of a 1% ethanolic solution of genipin and 10 g of PCL microspheres (300–425 μm range), and used to cast a first layer that was then left to partially cross-link for 2h, before a second layer composed of chitosan alone (without nHA) containing PCL microspheres (180–300 μm range) was cast on top. After crosslinking overnight and freezing for 24h at -20°C , the material was freeze-dried and dehydrated for at least 48h.

Scaffolds were then cut using a cork-borer, immersed for 4h in 2.5% alcoholic potassium hydroxide at 50°C to remove the PCL microspheres, and washed first in methanol then with distilled water. Finally, scaffolds were immersed in 1% sodium borohydride/water solution for 24h, washed in distilled water and then freeze-dried again for 48h. The resulting scaffolds were thus constituted of a bottom phase (subchondral) containing 70% w/w of nHA and pore size of $275 \pm 32 \mu\text{m}$, and a top phase (cartilage-like) made of chitosan alone with pore size of $160 \pm 12 \mu\text{m}$.