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Fabrication and development of artificial osteochondral constructs based on cancellous bone/chitosan-gelatin hydrogel hybrid scaffold

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Porcine cancellous bones and chitosan/gelatin hydrogel scaffolds were used as substitutes to mimic bone and cartilage. The porosity and distribution of pore size in porcine bone were measured and the degradation ratio and swelling ratio were also determined *in vitro*. Surface morphology was analyzed with SEM. The physicochemical properties and the composition were tested by using an infrared instrument. A double layer composite scaffold was constructed via seeding adipose-derived stem cells (ADSCs) induced to chondrocytes and osteoblasts, followed by inoculation in cancellous bones and hydrogel scaffolds. Cell proliferation was assessed through Dead/Live staining and an IpWin5 software. Cell growth, adhesion and formation of extracellular matrix in composite scaffolds blank cancellous bones or hydrogel scaffolds were also analyzed. SEM analysis revealed a super porous internal structure of cancellous bone scaffolds and pore size was measured at an average of $410 \pm 59 \mu\text{m}$ while porosity was recorded at $70.6 \pm 1.7\%$. In hydrogel scaffold, the average pore size was $117 \pm 21 \mu\text{m}$ and the porosity and swelling rate were $83.4 \pm 0.8\%$ and $362.0 \pm 2.4\%$, respectively. Furthermore, the remaining hydrogel weighed $80.76 \pm 1.6\%$ of the original dry weight after 6 weeks of hydration. In summary, the cancellous bone and hydrogel composite scaffold is a promising biomaterial which shows an essential physical performance and strength with excellent osteochondral tissue interaction *in situ*. ADSCs are a suitable cell source for osteochondral composite reconstruction. Moreover, the bi-layered scaffold significantly enhanced cell proliferation compared to the cells seeded on either single scaffold. Therefore, a bi-layered composite scaffold is an appropriate candidate for fabrication of osteochondral tissue.

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