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TISSUE ENGINEERING OF OSTEOCHONDRAL PLUGS USING HUMAN MESENCHYMAL STEM CELLS AND SILK SCAFFOLDS

In the past decade, bone and cartilage grafting based on tissue engineering from chondrocytes and osteoblasts, respectively, has emerged as a viable alternative to biological and synthetic grafts. Although effective, engineering approaches using differentiated cells harvested from the patient (e.g. terminally differentiated chondrocytes from cartilage or osteoblasts from bone) require invasive methods and destruction of structure at the site of harvest. Furthermore, the engineering of ostechondral plugs would require the selective seeding of scaffolds with osteoblasts in the bone and chondrocytes in the cartilage zone of the engineered implant. Mesenchymal stem cells are an alternative to differentiated cells as they can differentiate into either chondrocytes or osteoblasts in presence of appropriate stimuli. These cells are easy to harvest from human bone marrow, and can be expanded from small aspirate volumes resulting in millions of cells. The potential for selective differentiation suggests the principal possibility to drive differentiation along different lineages on the same scaffold through locally presented growth factors. Another important consideration is the biomaterial component, which is a critical determinant of the ultimate success of the tissue–engineered graft. Three dimensional silk scaffolds possess all the necessary properties required in a bone graft, unique mechanical properties, and opportunities for tailoring of structure. They also offers bioinduction, biodegradability and biocompatibility [1,2]. We evaluated silk scaffolds for bone tissue engineering and compare it to collagen scaffolds.

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EXPERIMENTAL METHODS

Scaffold preparation

Silk scaffolds were prepared from cocoons (*B. mori*). Cocoons were boiled in aqueous Na_2CO_3, solubilized in LiBr solution and dialyzed. Silk was redissolved in HFIP. Granular NaCl was weighed in a Teflon container and silk/HFIP solution was added. HFIP was allowed to evaporate and NaCl/silk blocks were immersed in 90% (v/v) methanol for 30 minutes.

Mesenchymal stem cell isolation, tissue engineering

Cells were separated from total bone marrow by density gradient centrifugation, and suspended in expansion medium (DMEM, 10% FBS, 1 ng/ml bFGF). After cells reached 80% confluence they were trypsinized, replated and passage 2 (P2) cells, were used for the experiments. 5 x 10^6 cells were seeded on a scaffold, and the constructs were cultured in osteogenic differentiation medium (50 ug/ml ascorbic acid–2–phosphate, 10 nm dexamethasone, 7 mM b–glycerolphosphate, and 1 ug/ml BMP–2 in DMEM) for up to 4 weeks.

Biochemical analysis

DNA content was measured using the PicoGreen assay (Molecular Probes, Eugene, OR). For calcium content, samples were extracted twice with 5% trichloroacetic acid and o–cresolphthalein complexone (Sigma, St. Louis, MO) was added and measured spectrophotometrically at 575 nm. Alkaline phosphatase activity was measured using a biochemical assay from Sigma (St. Louis, MO). To measure the amount of GAG, samples (n = 3 – 4 per group and time point) were frozen, lyophilized, and digested for 15 hours at 56°C with 1 mg/cm^3 papain solution. GAG content was

determined spectrophotometrically (Perkin Elmer, Oak Bridge IL) at 525 nm following binding to the dimethylmethylene blue dye.

RNA isolation, real–time RT–PCR

RNA was isolated with Trizol and the RNeasy kit (Quiagen, Hilden, Germany) (2). RNA samples were reverse transcribed into cDNA using oligo (dT)–selection (Superscript Preamplification System, Life Technologies, Gaithersburg, MD). Osteopontin, bone sialoprotein, bone morphogenic protein 2, type 2 collagen gene expression was quantified using the ABI Prism 7000 Real Time PCR system (Applied Biosystems, Foster City, CA). PCR reaction conditions were 2 min at 50°C, 10 min at 95°C, and then 50 cycles at 95°C for 15s, and 1 min at 60°C. The expression data were normalized to the expression of the housekeeping gene, glyceraldehyde–3–phosphate–dehydrogenase (GAPDH).

The visualization of spatial bone distribution, scaffolds were analyzed using a μCT20 imaging system (Scanco Medical, Bassersdorf, Switzerland). Scans were collected with a 34 μm in plane resolution and 125 μm slice thickness.

X–ray diffraction patterns of scaffolds before and after bone formation were obtained by means of Bruker D8 Discover X–ray diffractometer with GADDS multiwire area detector.

RESULTS AND DISCUSSION

After two weeks of incubation, cells started to deposit a mineralized matrix leading to calcification after 4 week. The overexpression of genes encoding for bone sialoprotein and osteopontin after 2 and 4 weeks accompanied the mineralization process. This was also measured for cbfa1 after 4 weeks.

Calcification clusters were connected giving a trabecular–like appearance of the bone structure. The tissue presented rod–like and plate–like structures and it appears that the mineralization clusters grew, at times, completely around the pores. μ–CT images showed very little mineralized tissue developed on the collagen scaffolds and the clusters only occasionally connected.

The crystalline nature of the deposited mineral was compared to poorly crystalline hydroxyapatite (pc HA), as present in bone (2). XRD analysis resulted in a similar spectrum as measured for the pc HA, suggesting that the deposited bone–like tissue was of similar nature as natural bone.

In chondrogenic medium, hMSC formed cartilaginous tissues on collagen and silk scaffolds, but the extent of chondrogenesis was substantially higher for hMSC cultured on silk as compared to collagen scaffolds. The deposition of glycosaminoglycan (GAG) and type II collagen and the expression of type II collagen mRNA were all higher for hMSC cultured on silk than on collagen scaffolds. Taken together, these results suggest that silk scaffolds are particularly suitable for tissue engineering of cartilage starting from hMSC, presumably due to their high porosity, slow biodegradation and structural integrity.

CONCLUSION

Together with its unique mechanical properties among natural fibres silks are a promising and new class of biomaterials for bone and cartilage tissue engineering. Mineralization advanced better on the silks compared to the collagen and so did the deposition of a cartilaginous matrix. Furthermore, the mineralized tissue had a trabecular–like appearance, thereby offering the opportunity to engineer complex bone geometries. These approaches demonstrates the principal feasibility to engineer ostechondral plugs on silks.

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