Striking The Balance Between Optimal Cell Response And Enhanced Tissue Repair In A 3D Multi-Layered Scaffold For Cartilage Repair

Valerie Barron[1], Martin Neary[1], Grace O'Malley[1], Niall Rooney[2], Frank Barry[1], Mary Murphy[1]
[1]Regenerative Medicine Institute, National University of Ireland, Galway/IE, [2]Proxy Biomedical Ltd., Galway/IE

Statement of Purpose: Cartilage is a complex multi-layered, zonally organized, aneural, avascular, poroelastic composite structure compromised of chondrocytes embedded in a water based glycosaminoglycan matrix reinforced with collagen fibers. Once damaged by osteoarthritis or trauma, self-repair/regeneration is limited. Current medical treatments involve microfracture techniques, autologous chondrocyte implantation or matrix assisted chondrocyte implantation, which stimulate fibrous cartilage formation. Although in the shorter term, these techniques improve mobility and alleviate pain, fibrous cartilage does not possess the optimal biological and mechanical properties to provide a long-term solution. The creation of a tissue engineering solution for such a multifactorial problem, requires selecting a material and design that delicately balances cell response, mechanical stability, degradation inflammation, integration and neo-tissue formation. Over the past 20 years, many research efforts have focused on 3D structures with mechanical properties in the MPa range, that address the functional mechanical property requirements of cartilage in articular joints at a macroscale and hydrogels with mechanical properties in the kPa region, which address the cell property requirements at a micro-level. However, there are no optimal biomaterials or designs that adequately address all of these materials property requirements. To this end, a 3D, slowly degrading, polylactic acid poly-ε-caprolactone (PLCL)/hyaluronic acid composite construct was developed; the cell response was characterized in vitro, the biocompatibility evaluated in vivo and the cell distribution optimized for enhanced cell-cell interaction for chondrogenesis.

Methods: Initially, a PLCL template based on the cell orientation and distribution of cells in native hyaline cartilage was produced by laser ablation in combination with thermal crimping to create a 3D porous substrate with a functionally graded pore structure. The 3D architecture was examined using scanning electron microscopy (Hitachi S-4700, UK) and microCT, from which the porosity was measured according to ASTM 2450-10. The compressive modulus was determined according to ASTM-F2450 using a load cell of 100N and a cross-head speed of 10mm/min (Zwick, UK). Cytotoxicity test methods were conducted in accordance with ISO 10993-12. An AlamarBlue™ assay (Molecular Probes) was employed to examine the metabolic activity of human mesenchymal stem cells grown in the presence of the scaffold for 72 hours by measuring the fluorescence intensity (530nm excitation/590 nm emission). Cell number was also assessed using a PicoGreen dsDNA quantification fluorescence assay (Molecular Probes) (485nm excitation/535 nm emission) on a plate reader. The biocompatibility of the PLCL structure was examined in an osteochondral defect in male White New Zealand rabbits in accordance with local ethical guidelines and approval, with 3 groups, empty defect (n=3), empty scaffold (n=6) and mesenchymal stem cell (MSC) seeded scaffold (n=6). After 4 weeks, the tissue was harvested, fixed in formalin, decalcified in Surgipath II, embedded in paraffin and sectioned at 5-micron intervals through the center of the defect. Toluidine blue staining was employed to evaluate inflammation and cellularity. Upon optimization of the cytotoxicity and biocompatibility of the PLCL construct, the optimal cell seeding conditions for *in vivo* repair was assessed *in vitro*. MSC were encapsulated in a hyaluronic acid (HA) gel using cell seeding densities from 25 X 10^6 to 60 X 10^6 cells/ml. After 24h in serum-free medium, the number of cells remaining in the composite scaffold was assessed using a PicoGreen assay, while the cell distribution throughout the scaffold was assessed using SEM.

Results: As shown in Figure 1a, a 3D PLCL structure was created with a porosity of 35%, pore dimensions increasing from 180μm in diameter on the top layer to 180 μm X 600μm on the bottom layer and compressive modulus of 10MPa. *In vitro*, no adverse affect on cell metabolic activity or cell number was observed for MSC grown in the presence of the construct.

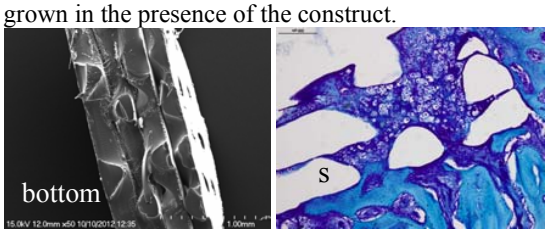

Figure 1(a) SEM image showing 3D functionally graded pore structure and (b) representative image showing in vivo biocompatibility where s is scaffold strut and n is neo-tissue formation

The *in vivo* evaluation revealed that the biomaterial was biocompatible as evidenced by the absence of any signs of inflammation or giant cells. More excitingly, after 4 weeks, there was evidence of neo-tissue formation with chondrocytes visible in the MSC-seeded scaffolds (Figure 1b). Using the HA gel, it was found that the optimal cell seeding density for cell-cell interaction for *in vitro* chondrogenesis was 60X10^6 cells/ml, which agrees well with previous studies using MSC-seeded hydrogels for cartilage[1] but with the added advantage of having the MPa-level compressive properties for functional support and kPa-level properties for cell-cell interaction.

Conclusions: In conclusion, a 3D composite structure was designed and developed to create an optimal cell environment in combination with functional mechanical properties for cartilage. The true repair potential of which will be investigated in a large animal model in future studies.

References:

1. Erickson, I.E., Acta Biomater 8, 3027, 2012.