TMJ tissue engineering: From the disc to the condyle

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ABSTRACT

The field of TMJ tissue engineering is blossoming, a field that was once far behind orthopaedic tissue engineering is rapidly gaining ground. Both TMJ disc and TMJ condyle tissue engineering efforts present unique challenges and will ultimately be necessary to regenerate TMJs for patients suffering from severe disorders. The TMJ disc is a fibrocartilaginous structure with complex attachments, where tensile mechanical integrity is a crucial design requirement. The TMJ condyle presents the challenge of engineering both bone and cartilage. Its cartilage is a fibrocartilage with four distinct zones, and the design requirement for mechanical integrity is the ability to resist compression and shear. We are focusing our efforts on the mandibular condyle, with efforts in all three areas of the tissue engineering triad: cell source, scaffold selection, and chemical signals. With regard to cell source, we are interested in comparing a traditional source of cartilage cells with an exciting new source in human umbilical cord matrix (HUCM) stem cells for engineering cartilage. The HUCM stem cells have shown promise in their ability to synthesize GAGs and collagen on poly(glycolic acid) scaffolds. Moreover, we have initial data to suggest that HUCM stem cells will be strong candidates for bone tissue engineering as well. Regarding scaffold selection, we are currently investigating new hydrogels and novel approaches to designing poly(lactic-co-glycolic acid) scaffolds. Our research in chemical signals has focused on the use of glucosamine, and on the use of proteoglycans along with growth factors. Our results have suggested that glucosamine may have a beneficial effect on TMJ condylar cartilage cells in culture, and effective concentration ranges are currently being investigated.

CELL SOURCE

HUCM stem cells develop from extraembryonic mesoderm, which forms the umbilical cord matrix (Mitchell *et al.*, 2003). They have been shown to be multipotential stem cells with properties between embryonic stem cells and adult stem cells. There is a lower incidence of graft vs. host disease than with bone marrow transplants, suggesting that umbilical cord cells have an innate mechanism to evade the immune system (Barker and Wagner, 2003). Currently, trials are underway using umbilical cord cells to treat a number of diseases. Only recently have HUCM stem cells been considered for tissue engineering, and for the most part have been focused on vascular tissue engineering. Recently, a German group reported that they were able to differentiate HUCM stem cells into cells with osteoblastic properties (Eblenkamp *et al.*, 2004). They suggested that HUCM stem cells are a promising source for cell-based therapies due to their ease of procurement and large supply. We intend to use this new cell source technology to engineer osteochondral constructs.

Cartilage Tissue Engineering With HUCM Stem Cells

Porcine TMJ condylar cartilage cells and HUCM cells were separately seeded onto PGA scaffolds for 6 days in a spinner flask. Spinner flasks for TMJ cells and HUCM cells contained control and chondrogenic medium, respectively. After seeding, constructs were then each cultured in either control medium or chondrogenic medium for an additional 4 wks. Although both groups were seeded at 5 million cells/mL, the HUCM cells were greater in number immediately after seeding and after 4 wks. After 4 wks, immunohistochemical staining demonstrated a strong presence of collagen I, and Saf-O/Fast green staining indicated a significant amount of GAG synthesis (Fig. 1). These results not only demonstrate the feasibility of using HUCM stem cells for chondrogenesis in 3D, but also suggest a superiority of these cells over a chondrogenic cell source.

Osteogenic Differentiation of HUCM Stem Cells

HUCM stem cells were cultured in monolayer for 12 days in control medium or osteogenic medium. Alizarin red staining demonstrated clear nodule formation and early osteogenic differentiation (Fig. 2). These encouraging results were the impetus for a

subsequent 3D investigation where HUCM stem cells were seeded directly onto PGA scaffolds and cultured in osteogenic medium for 1 wk. Again, alizarin red staining was positive, but more importantly, immunohistochemical analysis revealed an early presence of two osteogenic markers, alkaline phosphatase and osteopontin, in distinct isolated regions.

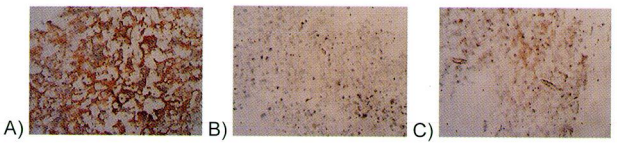

Figure 1: Saf-O/Fast green staining (200X), A) HUCM stem cells vs. B, C) condylar cartilage cells. Constructs were seeded for 6 days in a spinner flask (HUCM stem cells in chondrogenic medium, condylar cartilage cells in control medium), then cultured for 4 wks in static culture (A, B→control medium; C→chondrogenic medium).

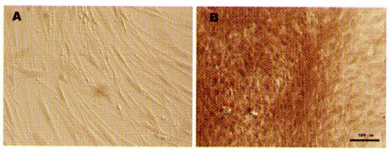

Figure 2: HUCM stem cells grown in A) control or B) osteogenic medium for 12 days, stained with alizarin red.

SCAFFOLD SELECTION

Microparticle-based scaffolds have recently incited enthusiasm in tissue engineering due to their ease of fabrication, the ability to discretely control particle physicochemical properties, and versatility for controlling the release kinetics of bioactive molecules. Microparticles fabricated from biodegradable polymers such as poly (glycolic acid) (PGA), poly(lactic acid) (PLA) or co-polymers of the two (PLGA) offer the added benefits of modulating degradation kinetics, and of FDA approval for use in humans. Chondrocytes and osteoblasts have been successfully cultured on scaffolds composed of PLGA microspheres containing active compounds such as BMP-2 and TGF-β (Elisseeff *et al.*, 2001; Lu *et al.*, 2001; Oldham *et al.*, 2000). With our collaborators, we have a unique ability to create uniform PLGA microspheres to facilitate production of tissue scaffolds with precisely reproducible physical features. Our goal with our present work is to control the spatial organization of encapsulated signal molecules, which we can then use to direct HUCM stem cell differentiation in a region-specific manner.

CHEMICAL SIGNALS

There is a plethora of evidence for the safety and efficacy of glucosamine and chondroitin sulfate for treating osteoarthritis, including several recent reports (Anderson *et al.*, 2005; Brief *et al.*, 2001; Bruyere *et al.*, 2004; Chalmers, 2005; Christgau *et al.*, 2004; Davenport, 2004; Einhorn, 2004; Nakamura *et al.*, 2004; Zerkak and Dougados, 2004). Successes with glucosamine were the impetus for the formation of the Glucosamine Arthritis Intervention Trial (GAIT), created in part with funding from the National Institute of Arthritis and Musculoskeletal and Skin Diseases. GAIT was a 24-wk placebo-controlled study of 1588 patients at 13 centers, with results being released in just the past few months, with the conclusion that glucosamine and chondroitin are effective in treating osteoarthritis. There is a strong possibility that glucosamine-chondroitin may provide for an effective new strategy in cartilage tissue engineering, which we are currently investigating.

Glucosamine in Monolayer Culture

In a 2-week monolayer study with confluent porcine ankle chondrocytes in 12-well plates, wells with only 0.1 mg/mL of glucosamine produced 20 ± 6 mg GAG/well compared to 11 ± 2 mg for the control (Fig 3).

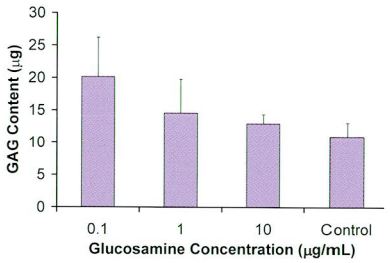

Figure 3: GAG content in monolayer cultures of chondrocytes in 2 weeks, based on varying concentration of glucosamine content. It is interesting to note that higher concentrations of glucosamine do not necessarily ensure a higher GAG content.

REFERENCES

Anderson JW, Nicolosi RJ, Borzelleca JF (2005). Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem Toxicol* 43(2):187-201.

Barker JN, Wagner JE (2003). Umbilical-cord blood transplantation for the treatment of cancer. *Nat Rev Cancer* 3(7):526-32.

Brief AA, Maurer SG, Di Cesare PE (2001). Use of glucosamine and chondroitin sulfate in the management of osteoarthritis. *J Am Acad Orthop Surg* 9(2):71-8.

Bruyere O, Pavelka K, Rovati LC, Deroisy R, Olejarova M, Gatterova J, Giacovelli G, Reginster JY

(2004). Glucosamine sulfate reduces osteoarthritis progression in postmenopausal women with knee osteoarthritis: evidence from two 3-year studies. *Menopause* 11(2):138-43.

Chalmers R (2005). Managing osteoarthritis of the knee: glucosamine-chondroitin should be prescribed by doctors. *Bmj* 330(7492):673.

Christgau S, Henrotin Y, Tanko LB, Rovati LC, Collette J, Bruyere O, Deroisy R, Reginster JY (2004). Osteoarthritic patients with high cartilage turnover show increased responsiveness to the cartilage protecting effects of glucosamine sulphate. *Clin Exp Rheumatol* 22(1):36-42.

Davenport G (2004). Rheumatology and musculoskeletal medicine. *Br J Gen Pract* 54(503):457-64.

Eblenkamp M, Aigner J, Hintermair J, Potthoff S, Hopfner U, Jacobs V, Niemeyer M, Wintermantel E (2004). [Umbilical cord stromal cells (UCSC). Cells featuring osteogenic differentiation potential]. *Orthopade* 33(12):1338-45.

Einhorn TA (2004). If it feels good, do it: use of glucosamine sulfate to prevent the progression of osteoarthritis in postmenopausal women. *Menopause* 11(2):134-5.

Elisseeff J, McIntosh W, Fu K, Blunk BT, Langer R (2001). Controlled-release of IGF-I and TGF-beta1 in a photopolymerizing hydrogel for cartilage tissue engineering. *J Orthop Res* 19(6):1098-104.

Lu L, Yaszemski MJ, Mikos AG (2001). TGF-beta1 release from biodegradable polymer microparticles: its effects on marrow stromal osteoblast function. *J Bone Joint Surg Am 83-A Suppl* 1(Pt 2):S82-91.

Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D (2003). Matrix Cells from Wharton's Jelly Form Neurons and Glia. *Stem Cells* 21(1):50-60.

Nakamura H, Shibakawa A, Tanaka M, Kato T, Nishioka K (2004). Effects of glucosamine hydrochloride on the production of prostaglandin E2, nitric oxide and metalloproteases by chondrocytes and synoviocytes in osteoarthritis. *Clin Exp Rheumatol* 22(3):293-9.

Oldham JB, Lu L, Zhu X, Porter BD, Hefferan TE, Larson DR, Currier BL, Mikos AG, Yaszemski MJ (2000). Biological activity of rhBMP-2 released from PLGA microspheres. *J Biomech Eng* 122(3):289-92.

Zerkak D, Dougados M (2004). The use of glucosamine therapy in osteoarthritis. *Curr Rheumatol Rep* 6(1):41-5.