Bioactive Glass (13-93) as a Subchondral Substrate for Tissue-Engineered Osteochondral Constructs

NTRODUCTION:
Bilayered tissue-engineered osteochondral (OC) grafts require the integration of two key components; a superficial layer consisting of articular cartilage cells encapsulated in a hydrated matrix that maintain the chondrocytic phenotype and an underlying layer comprising a stiffer, porous material to provide mechanical support and integration into the subchondral bone. Bioactive glasses (BG) are bioresorbable materials that have been shown to be osteoinductive and osteoconductive when used as scaffolding materials to fill bone defects. We have previously reported that BG13-93 has beneficial effects on cell-seeded constructs when used as a media supplement. The next step was to investigate whether this would translate when BG 13-93 is used a subchondral base for bilayered tissue-engineered constructs and lead to a gradient response in cartilage growth. Bilayered OC constructs were fabricated with chondrocyte-seeded agarose hydrogels bonded to a BG 13-93 base and cultured for up to 6 weeks.

METHODS:
BG fabrication: Using a polymer foam replication technique, BG with 13-93 composition wt%: 53SiO₂, 6Na₂O, 12K₂O, 5MgO, 20CaO, 4P₂O₅ Fig 1 was made into cylinders. The BG cylinders had a porosity of 85 ± 2% and pore sizes ranging from 100-500μm matching the microstructure of human trabecular bone.

Construct Fabrication: Chondrocytes were harvested from the carpometacarpal joints of juvenile calves and seeded into 2% agarose (Type VII, Sigma) at 30 million cells/mL. Chondrocyte-laden agarose was poured into pre-prepared molds and the bioactive glass substrates were pressed into the molten agarose. Cell-seeded agarose only constructs without a subchondral base served as controls (Fig 2), n=5 in each group at each time point. Constructs were cultured in serum-free, chemically-defined media supplemented with ascorbate, TGF-β3 (10 ng/mL) was added for the first 14 days of culture. Media was changed and collected every 48-72 h. Constructs were harvested on days 0, 14, 28 and 42. At harvest constructs were halved for biochemical analysis and histology/immunohistochemical evaluation.

Tissue Assessment: Constructs underwent protease K-digestion and were assayed for DNA, glycosaminoglycan (GAG) and collagen content standardized for wet weight. Culture Media Assessment: Culture media were collected every 2-3 days and assayed for GAG, collagen, nitric oxide (NO) and prostaglandin-E₂ (PGE₂). Histology: Constructs were fixed in formalin and paraffin embedded. Transverse sections of the agarose gel layer were taken. Haematoxylin and eosin (H&E) stain was used to assess tissue morphology, toluidine blue to assess proteoglycan and trichrome to assess collagen. Immunohistochemistry (IHC): Standard IHC techniques were used to subjectively analyze proteoglycan, collagen 1 (Col I) and collagen 2 (Col II) content. Statistical Analysis: A one-way ANOVA (α=0.05) with Tukey tests was used to compare outcome measures between groups.

RESULTS:
Osteochondral constructs with a BG-13-93 subchondral base had higher concentrations of GAG in the chondrocyte-seeded agarose gel layer (5.8%/w/w, comparable to native bovine wrist cartilage) and media when combined, compared to controls at a 6 week time point (Fig 3a, p<0.05).

There was a significantly higher concentration of PGE₂ in the media from BG constructs at all time points (Fig 3b, p<0.05) compared to controls without a difference in NO concentration. Upon observing IHC images for the distribution of the GAG, the BG constructs had a higher amount concentrated in the central region versus the periphery whereas controls had a lower distribution throughout but particularly in the central region (Fig 4 A1-B1 and A3-B3). The collagen content of the BG constructs overall was significantly lower compared to controls (p<0.05) however when normalized to the number of cells, there was no significant difference. BG constructs did not have a homogenous distribution of collagen compared to controls particularly in the central portion of the tissue (Fig 4 A2-B2). IHC showed that BG did create a collagen type I rim on the outer surface of the construct, not observed with controls (Fig 4 A4-B4), but did not have a high amount of collagen type 2 (Fig 4 B5). The pH of the media from BG constructs at a 6-week time point was more alkaline (7.94) compared to controls (7.67).

DISCUSSION:
We investigated the biochemical effects of BG-13-93 as a subchondral substrate for bilayered tissue engineered OC constructs. BG increased GAG production in the cell-seeded agarose gel layer and media. Collagen type 1 was increased in the periphery of the construct (at the expense of GAG). However collagen type 2 deposition was not improved compared to controls. As previously described, a possible mechanism is that as BG 13-93 degrades in the media it could be releasing constituents that alkalinize the media enhancing extracellular matrix production. These constituents could also lead to cellular production of inflammatory mediators such as PGE₂ which could be significant in an in vivo situation. Although BG 13-93 improved extracellular matrix deposition when used as a subchondral substrate the type of cartilage produced does not resemble native cartilage in collagen content. The present study does however lend further credence to the use of BG 13-93 as a media supplement for neo-cartilage formation. Future studies could aim to identify the types of GAG produced, its relationship to the pH and ions in the media and the spatial variation in distribution away from the BG by taking axial rather than transverse sections.