

BIOMECHANICAL EVALUATION OF CHONDROCYTE-SEEDED SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

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INTRODUCTION

Various 3D matrices have been employed in the attempt to develop tissue-engineered cartilage, such as type I and type II collagen-based gels or sponges, fibrin glue, polysaccharide hydrogels, membranes made of non woven fibers of poly-L-lactic acid (PLLA), poly-glycolic acid (PGA), hyaluronic acid (HA) and various polyetherurethane foams. These artificial matrices are designed to progressively degrade while their mechanical function is replaced by the extra cellular matrix (ECM) synthesized by the living cells.

Despite many advances in scaffold development, to date all attempts to develop tissue-engineered cartilage have led to materials with biochemical composition and mechanical properties far inferior to those of the natural tissue. Although it may be unnecessary to develop properties identical to natural cartilage, as the graft may remodel once implanted, there is currently no evidence that tissue-engineered cartilage can support the loads acting on diarthrodial joints after surgical implantation [1].

We have evaluated the biomechanical properties of cellular constructs obtained using three different scaffold materials: fibrin glue, a collagen sponge and a polyurethane foam. The scaffolds were seeded with human chondrocytes and were cultured *in vitro* for two weeks. The cellular constructs were tested in dynamic shear on a rheometer.

MATERIALS AND METHODS

Construct preparation

Biopsies of articular cartilage were harvested aseptically from four patients with a history of trauma. Chondrocytes were isolated by digestion with type II collagenase and the cells were expanded in complete culture medium. After reaching semi-confluence, some cells were trypsinised and directly seeded on the scaffolds, others were collected by centrifugation, stored in liquid nitrogen and subsequently seeded for a second expansion.

Three scaffold materials were evaluated: fibrin glue, a collagen sponge and a polyurethane foam. Fibrin glue constructs were obtained using the commercial surgical sealant Tissucol® 2,0 (Baxter AG,

Vienna, Austria). A pellet of $35 \cdot 10^6$ chondrocytes was suspended in a solution A, containing human fibrinogen and a protease inhibitor. Solution A was mixed with a solution B, containing human thrombin and CaCl_2 . The polymerizing gel was extruded into a sterile silicon tube of 6.35 mm in diameter. The tube was cut in 5 mm thick slices and the cylindrical constructs were extracted, weighted and positioned in a culture cluster.

Collagen constructs were produced using the commercial sponge of type I collagen Antema® (Opocrin S.p.A., Modena, Italy). Sterile 7x3.5 cm pads of thickness 2.5 mm and a nominal pore size of 200 μm were punched into circular 16-mm diameter discs. Each disc was positioned in a culture cluster and seeded with $5 \cdot 10^6$ cells. The seeded discs were incubated for 3 hours at 37° to allow for cell adhesion.

The third scaffold evaluated is DegraPol®, a biodegradable polyetherurethane foam, developed at the Swiss Federal Institute of Technology (ETH) in Zurich. This block copolymer contains crystalline domains of Poly[(R)-3-hydroxybutyric acid] (PHB) and amorphous domains of poly(glycolide-co-caprolactone). The polymer is processed into open porous structures (foams) by a freezing-precipitation technique. The scaffolds employed in our study were sterile 16-mm diameter discs of 2 mm thickness and of nominal pore size 100 μm . The discs were wetted in medium for 2 hours at 37° under partial vacuum, because of their hydrophobic nature, were positioned in a culture cluster and seeded with the cells. Three independent experiments were conducted on these foams, at different cell seeding densities: $7 \cdot 10^6$, $20 \cdot 10^6$ and $22 \cdot 10^6$ cells/disk. The seeded discs were incubated for 3 hours to allow for cell adhesion.

One DegraPol® construct was placed in a closed-loop recirculation system [2]. Both the bioreactor and the culture clusters were incubated for 2 weeks with medium completely replaced every 2 days.

Biomechanical testing

For each scaffold material, cylindrical samples were punched from the constructs at days 1 and 14 of culture and from cell-free controls at day 1 of culture. Cartilage samples were also prepared for

comparative testing, those were obtained from a fractured human femoral head. All the samples were stored in culture medium and tested in dynamic shear within 4 hours, using a Bohlin CVO 120 controlled stress rheometer (Bohlin Rheology AB, Lund, Sweden), with a plate-to-plate testing configuration.

decreases the constructs' G' of one order of magnitude. The DegraPol foam (Fig.01, right) shows higher G' values, if compared to the other scaffolds. Here, G' decreases after cell seeding and remains stable up to two weeks of *in vitro* cultivation. All the constructs show phase angle values, δ , in the order of those measured for natural articular cartilage at physiological frequencies (Fig.01, bottom).

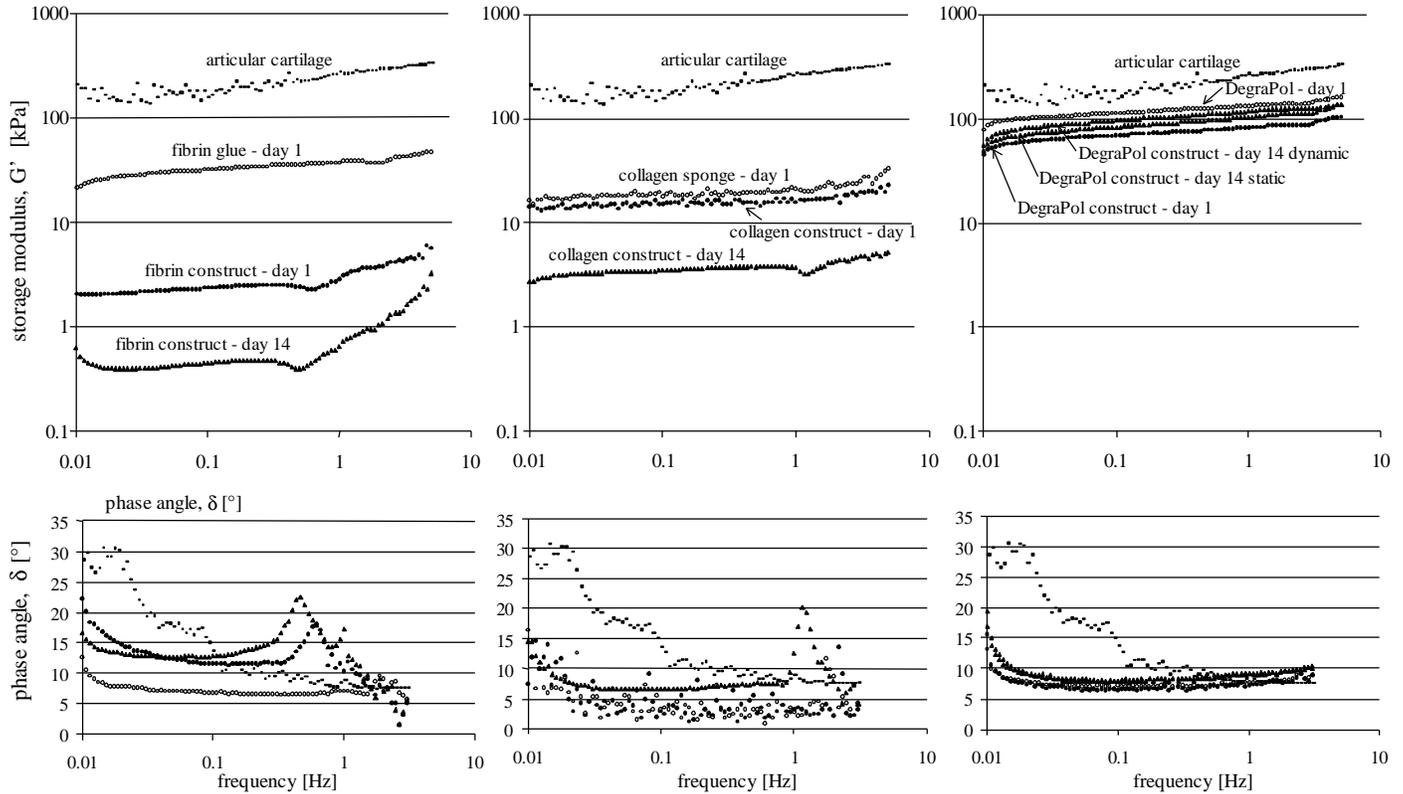


Figure 1. Results of the dynamic shear testing

The rheometer applies a controlled sinusoidal torque to the sample and a position sensor measures its angular deflection. The signals are processed and the material's complex shear modulus, G^* , is calculated [3]. For each material, one sample was tested by imposing increasing shear stresses at a 1 Hz frequency (stress sweep). Other two samples were tested by imposing a fixed shear stress level at increasing frequencies, in the range $10^{-2} \div 5$ Hz (frequency sweep).

RESULTS AND DISCUSSION

In this study the material behavior was described using two parameters, calculated from the complex shear modulus, G^* , in the frequency sweeps. The two parameters are the storage modulus, G' , which measures the purely elastic response of the material, and the phase angle, δ , related to the degree of viscous to elastic character of the material.

The values of G' and δ obtained from the measurements are shown in Figure 1 for all the samples. Articular cartilage generally shows G' values higher than all the other samples tested, in the order of 0.2 MPa at a 1 Hz frequency. In fibrin constructs (Fig.01, left), cell seeding induces a decrease in G' of one order of magnitude and a further significant decrease is seen after two-week of *in vitro* cultivation. In the collagen sponge (Fig.01, middle) cell seeding does not significantly affect the G' , while the two-week cultivation

Dynamic shear testing allows to quantify flow-independent viscoelastic properties of strong gels, consisting of a solid network structure in a fluid phase. This testing method has a great potential as a tool for the design and development of tissue-engineered constructs. It allows the comparative evaluation of different scaffold materials, with respect to their biomechanical properties and degradation rate. In cellular constructs, dynamic shear testing allows to correlate the biomechanical properties to the microstructure and to the biochemical composition of the constructs at different culture stages.

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