Mechanical Loading of Hydrogels with a Multi-specimen Fixture

The Challenge:

Apply Cyclic Compressive Loading to Multiple Specimens Under Sterile Conditions

Background

Degeneration of cartilage within joints typically causes pain due to primary osteoarthritis, injury or disease. Unfortunately, adult cartilage has very limited ability to self-heal, and further degeneration may occur. The number of patients that suffer from this illness keeps growing every year, and there is a rising need for effective treatment.

Figure 1 - Bose® ElectroForce® 5500 Test Instrument

Currently, donor site morbidity and the limited availability of autologous osteochondral plugs limit the usefulness of mosaicplasty and autologous chondrocyte implantation. In response, tissue engineering bioreactors have been developed to produce cartilage substitutes, in which isolated autologous chondrocytes are seeded. These engineered alternatives provide at least a minimal level of function immediately following implantation. If chondrocytes experience external loading during their development, they secrete cartilaginous proteins and organize into the correct morphology. Thus, bioreactors that are capable of applying consistent and accurate mechanical loading often produce significantly better results compared to static culture methods. In this experiment, the Bose® 5500 test system equipped with a multi-specimen fixture (Figure 1) was used to deliver this mechanical stimuli to hydrogels used as model cartilage substitutes.

Meeting the Challenge

During the past decade, there have been remarkable advances in growing cartilage in vitro, but transferring this technology to a clinical setting remains problematic. As of now, there is no current method that can mass-produce the neccessary biomaterials for artificial cartilage.

The Bose ElectroForce® 5500 test instrument with a multi-specimen fixture, which can be used in combination with a 24-well plate containing the disc constructs (Figure 2), is ideal for mechanical studies of tissue-engineered cartilage. It not only applies precise axial loading, but also performs multi-specimen testing, which significantly reduces labor.

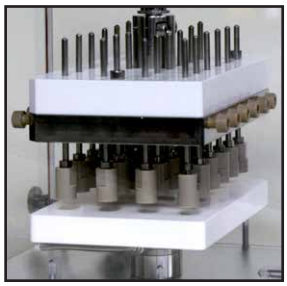

Figure 2 - 24-Well Plate Multi-specimen Fixture

Materials and Methods

Figure 3 - Hydrogel Specimens in 24-Well Plates

PEG hydrogels sheets (4" x 4") were purchased from Medline Industries. Hydrogel specimens, punched from the hydrogel sheet, were 12 mm in diameter and 1.2 mm in height (Figure 3).

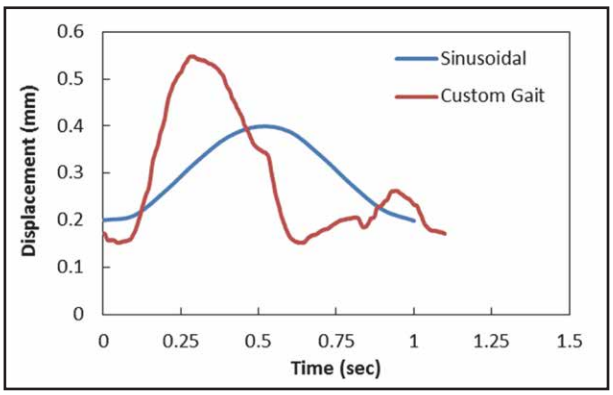

Figure 4 - Sinusoidal and Custom Gait Waveforms

In this study, the ElectroForce® 5500 test instrument equipped with a 200 N load cell was used to load the samples, and all the specimens were soaked in phosphate buffered saline (PBS) during testing. Both single and 24 specimens were tested under a displacement controlled sinusoidal waveform and a custom gait waveform (Table 1, Figure 4). Load and displacement data were recorded.

Table 1. Experimental Conditions

1 Specimen / Loaded	Sinusoidal waveform: data in Fig. 6a Custom gait waveform: data in Fig. 6c
24 Specimens / Loaded	Sinusoidal waveform: data in Fig. 6b Custom gait waveform: data in Fig. 6d
12 Specimens / Loaded & 12 Specimens / Unloaded	Custom gait waveform (Data not shown, similar to Fig. 6c) Three samples each from loaded and unloaded groups tested to failure with single pair of platens: data in Fig. 7

The effect of the custom waveform on the specimen mechanical properties was also evaluated. Twelve specimens were loaded with the custom waveform, while the other twelve were the unloaded controls using the 24-well fixture. Then, three specimens from each group (loaded and unloaded) were tested in compression by the ElectroForce 5500 system with a 200 N load cell and a pair of 25 mm diameter solid platens (Figure 5).

Figure 5 - Hydrogel Specimens Loaded Between Compression Platens

Results

Under the same sinusoidal waveform, the cumulative load of the 24 samples (max. 13.6 N, Figure 6b) was 22 times higher than that of the single sample (max. 0.62 N, Figure 6a). This result indicates that the multi-specimen fixture allows for similar preload of samples prior to loading and the application of the same displacement waveform on all samples.

Similar results were obtained from the custom waveform experiments (Figure 6c and 6d). Finally, a higher fracture load was observed in unloaded specimens compared to loaded samples (Figure 7), which indicates that mechanical loading with the custom waveform has a significant effect on the hydrogel's mechanical properties.

Summary

The Bose® ElectroForce® 5500 test instrument combined with a multi-specimen compressive fixture is capable of providing a highly controlled mechanical loading environment for disc-shaped constructs.

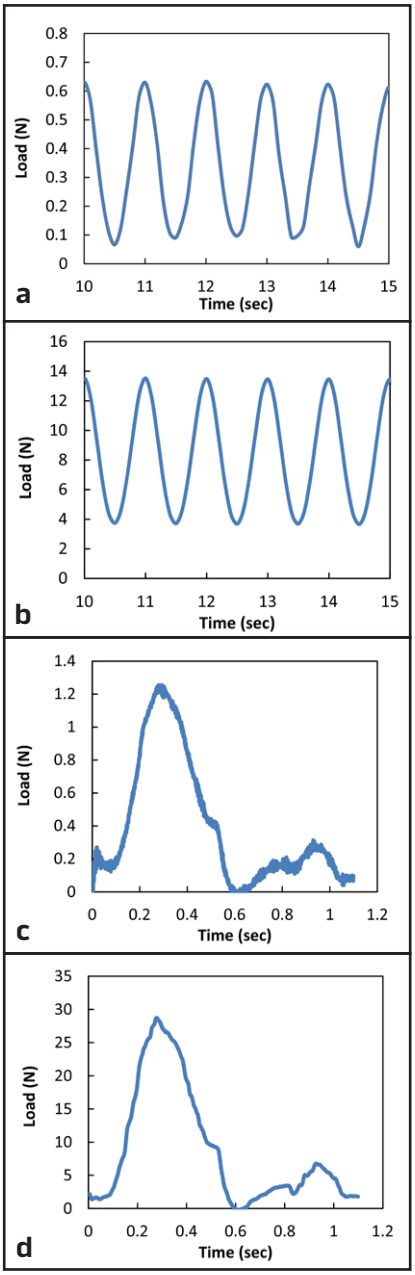

Figure 6 - Load Results: a) Single Specimen, Sinusoidal Waveform b) 24 Specimens, Sinusoidal Waveform c) Single Specimen, Custom Waveform d) 24 Specimens, Custom Waveforms

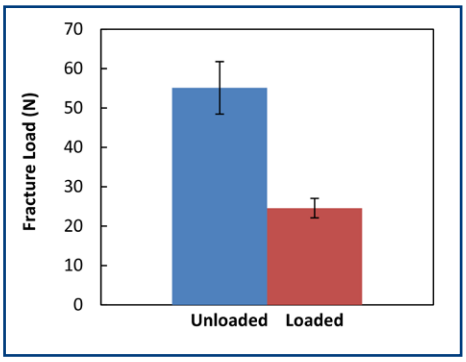

Figure 7 - Fracture Loads of Unloaded and Loaded Specimens

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