Knee cartilage mechanical characterization to inform specimen-specific finite element models

1. Motivation and objectives

Articular cartilage is a complex multilayer, multi-component structure. The underlying components have widely different material properties. Mechanical response of cartilage is also tied to fluid flow in the tissue. This structure provides load bearing and shock absorption capabilities. The biomechanical characteristics of the tissue depend on the multiphasic nature[1]. Understanding of cartilage mechanics is essential for development or treatments options and preventative measures for pathologies.

Finite element analysis is being widely used to further the understanding of mechanics of biological structures and assist in development of said treatment options and measures. An important concern is whether specimen / patient specific information is needed in developing these models and to what extent and detail, depending on the question at hand. We are interested in development of finite element models of the knee joint and an important component of that is the articular cartilage. This document provides an overview of the work that we have done so far to efficiently and effectively characterize specimen specific knee cartilage material properties and where it fits in the envelope of existing cartilage mechanics literature.

2. *Literature review*

Information extracted for cartilage mechanical testing from studies with varied goals and objectives.

Table 1: Summary of selected studies focused on articular cartilage mechanical characterization

Study	Goal	Specimen source	Sample location	Specimen age and/or gender	# of specimens and samples	Sample dimensions	Protocol	Test type	Output/results/ Material properties
Morel et al, 2005 [2]	Effects of injurious compression	Bovine	Humeral heads	18 month old	Samples = 55	4mm diameter bone, 2.7 mm dia cart plugs, full thickness	Compression applied at 0.07/s and 0.00007/s upto 14 Mpa.	Unconfined Compression	Swelling / thickness
Langelier et. al., [3]	Assess strain and strain rate dependent stiffening	Bovine	Humeral heads	1-2 yr old	4 discs /speed	3 mm disc, varying thickness (<1mm)	6g tare load, compression-hold-release (12.5; 25; 50; 100; 150; 200; 250; 300 mm) using 1 of 3 ramp speeds (0.5; 5; 50 mm/s	Unconfined compression	equilibrium stiffness of the first test ramp (12.5 mm) : 0.40– 0.61 MPa for low strain rate compression, 0.30–0.56 MPa for moderate strain rate (fresh and frozen specimens) compression and 0.33–0.56 MPa for high strain rate compression.
Toyras et. al., [4]	Determine Young's modulus and thickness	Bovine	Humeral heads	-	7	For unconfined compression – 3 mm diameter	Unconfined compression – stepwise stress relaxation.5 steps, each 4% of cartilage thickness, speed 2 mm/s ,relaxation time of 13 min between each step. The equilibrium stress-strain data in the linear range during the three last steps used to determine the equilibrium Young's modulus	Ultrasound, indentation and unconfined compression	Young's modulus : 0.1- 1.6 MPa
Lai et. al., [5]	Investigate depth varying compressive strains	Bovine	Tibial articular cartilage	Young	72 total samples	4 mm diameter / 1500 micron thick (bottom removed)	compressed at 1 mm/s to 5, 10, 15, and 20% strain, relax for 20 min at each offset.	Unconfined compression	Average equilibrium compressive modulus: 0.447 (0.363, 0.531) MPa
Boschetti et. al., [6]	Properties of cartilage under compression	Human	Femoral head	73-M, 75-M, 81-F	3 specimens, 33 discs total	5 mm diameter, 650 micron thick, 3 layers	1.5 MPa for 1800s	Confined and unconfined compression	Confined : Average aggregate modulus: 0.25, 0.38,0.5 MPa. Unconfined : Average Equilibrium modulus: 0.24, 0.38,0.5 MPa
Jurvelin et; al., [7]	Mechanical anisotropy or	Human	Patellofemoral groove	3 females, 7 males,	10 specimens	1.7 mm diameter, 1 mm thick	1 micron, 1 micron/s up to 20 % strain. Relaxation limit: 0.1g /min	Confined and unconfined	Aggregate modulus: 0.845±0.383 MPa

	knee cartilage			aged 23–50 years				compression	Young's modulus: 0.581±0.168 MPa
Treppo et. al., [8]	Biomechanical comparison of knee-ankle cartilage pair	Human	Tibiofemoral, patellofemoral surfaces	-	8 specimens, 10 samples from each joint	3 mm disc , top 1mm slices	Static compressive strain 5-10% up to 25 %	Confined compression	Femur (n=48): Average aggregate modulus = ~ 0.6 MPa, Tibial plateau (n=31): ~ 0.5 MPa
Armstrong et. al., [9]	Variation in properties with age, water content, degeneration	Human	Patella	56.4 yrs (SD: 19.13)	103 samples	6.35 mm diameter	0.1 MPa constant stress	Creep confined compression	Modulus: 0.79 MPa (0.36)
Charlebois et al., [10]	Age and depth dependent tensile properties	Bovine	Humeral heads	< 12 months - adult	26 specimens	6-8 mm wide, 2 cm long, ~ 5 mm thick	5 ramp of 2% strain up to 80 microns(6 micron/second) . 48 hour long tests	Tensile	modulus for the last step : 10.1 (3.3) MPa for young, 18.7(5.9) MPa for adolescent, and 28.3(16.6) MPa for adult.
Froimson et al., [11]	Cartilage surface fibrilation effects	Human	Patellofemoral joint	18-41 yrs	17 specimens, 10 sites each	1.5 mm dia indentor	- --	Biphasic indentation	Average aggregate modulus: 0.42 MPa (Patella) 0.6 MPa (groove)
Bellucchi et al., [12]	Articular cartilage under tensile load	Human	Patellofemoral and tibiofemoral joints	48 yrs	1 specimen, 16 samples, 72 tensile specimens	0.2 mm thick, 1mm by 9 mm, multiple depths	1.5 – 3 MPa maximum stress	Cyclic Tensile	Cycles to failure : 20 – 1.5 * 10^6
Gao et al., [13]	Depth and rate dependent cartilage behavior	Porcine	trochlea sites of three joints of knee	8 months	18 samples	5.5 by 4 mm, 2 mm thick	stress rates0.0045, 0.045, 0.225 Mpa/s, constant compressive stress levels of 0.1, 0.5 and 1 MPa , creep time: 60 min	Unconfined compression creep	Young's modulus: at 0.0045 MPa /s: 1.4-4.8 MPa (superficial – deep layer) at 0.045 MPa /s: 5-6.5 MPa (superficial – deep layer)
Changoor et al., [14]	Effects of freezing	Bovine	Knee joint	~ 6 months	8 sites/ 4 samples each	3 mm diameter	ramps of 2% strain applied at a rate of 0.4% strain/s, relax until the load decay was 0.01 g/min	Unconfined compression	Fresh/ non frozen samples: fibril modulus, day 1: average 16.5 MPa (n=8) matrix modulus, day 1: Average 0.7 MPa (n=8)
Thambyah et al., [15]	Properties of cartilage covered by meniscus	Human	Tibial plateau	62-70 yrs	7 specimens	Load bearing and non load bearing regions	axial compression at a constant load of 0.5 N (0.6 MPa), with the use of a 1-mm diameter indenter, 60s hold.	indentation	Lat plateau not covered by meniscus: 2.13 (0.74) MPa Med plateau not covered by meniscus: 3.51 (1.42) MPa Lat plateau covered by meniscus: 3.77 (1.25) MPa Med plateau covered by meniscus: 5.13 (1.91) MPa
Bursac et al., [16]	Assess confined and unconfined stress relaxation	Bovine	Patellar groove	3-4 weeks old	3 specimens	4.5 mm diameter, 3.5 mm thick	Steps of 3% ramp up to 15 % strain (0.115 micron /s)	Confined and unconfined compression	Aggregate modulus, MPa at 6%,9%,12%,15% strain: 1.72 (0.72), 1.23 (0.4), 0.97 (0.2),

	with transversely isotropic model								0.79 (0.12). Young's modulus, MPa at 6%,9%,12%,15% strain: 1.5 (0.5), 1.09 (0.43), 0.74 (0.28), 0.68 (0.13).
Nguyen et al., [17]	Swelling influences on mechanics in compression and shear	Bovine	Femoral condyles	Immature	50 samples	4mm diameter, 2 mm thick mid-substance	5%, 10%, 15%, and 20% strain at 0.1%/s, 20 min relaxation	Unconfined compression	1.47 ± 0.13 MPa
G. E. Kempson [18]	Relationship between tensile properties and age	Human	Femoral condyles	8-91 yrs		3 mm by 0.5 mm, 200 micron thick	(Protocol unclear) tensile stiffness determined from the gradient to the stress strain curve at 2 levels of stress, namely, 5 MN/m2 and 10 MN/m2.	Tensile test	Tensile properties of human articular cartilage from the femoral condyles of the knee deteriorated with increasing age
Athanasiou et al., [19]	Interspecies comparison of mechanical properties	Human and other	Distal femoral cartilage	-	4 specimens	1.5 mm diameter indentor	Tare load (0.03-0.04 N), 0.1961 N load, 3 hour creep relaxation	Indentation	Lateral condyle, modulus= 0.701 ± 0.228 MPa Medial condyle, modulus: 0.588 ± 0.114 MPa Patellar groove, modulus: 0.530 ± 0.094 MPa
Robinson et al., [20]	Normal vs osteoarthitic (OA) cartilage	Human	Tibiofemoral joint	10 specimens OA (4 male,6 female, 69.7± 9.3 yrs) , 3 specimens normal (1 male, 2 female, 59.1 ±7.2 yrs)	51 samples OA, 43 sample normal	3 mm diameter osteochondral plugs	< 0.3 N tare load, 30% strain at 20%/s (estimated walking rate)	Unconfined compression	Tibia control: 447.1±218.7 MPa Tibia OA: 375.5±371.7 MPa Femur control:431.7±486.1 MPa Femur OA: 469.2±438.7 MPa
Deneweth et al., [21]	Tibial plateau heterogeneity	Human	Tibial plateau	All female , 18-55 yrs	8 specimens	4 mm diameter	0.2 N tare load, 10 cycles at 100%/s to 20% strain. 3 trials of 100%/s to 20% strain rapidly return to 0% strain. (~ stance phase of gait)	Unconfined compression	tangent modulus to the stress–strain curve at 10% strain for various locations: 1-80 MPa
Korhonen et al., [22]	Equlibrium response at confined, unconfined compression and indentation	Bovine	Humeral head	-	26 samples	3.7 mm diameter for compression	stepwise stress–relaxation tests (each step 5% strain at velocity 1 mm/s) up to a strain of 20%. Complete relaxation was a relaxation rate <100 Pa/min.	Confined and unconfined compression	Patella modulus, unconfined : 0.57±0.17 MPa Femur modulus, unconfined: 0.31±0.18 MPa Patella modulus, confined : 0.62±0.19 MPa

									Femur modulus, confined: 0.34±0.17 MPa
Shepherd et al., [23]	Instantaneous compressive modulus	Human	Tibiofemoral joint	65.1 (14.3) yrs. 3 male , 8 female	11 specimens	1.58 mm diameter	Cartilage loaded within 30 ms (compressed spring in apparatus applies the full load (10.5 N) in 10–15 ms.)	Indentation	Sample set: Compressive modulus, patella: 5.9 (2.1) MPa. Femur: 8.3 (4.2) MPa
Nissi et al., [24]	Estimation of mechanical properties using MRI	Human and other	Tibiofemoral joint	24-78 yrs	12 specimens	5 mm diameter	Compression: 10% prestrain, 10% strain with 2 mm/s ramp rate and 40 min of relaxation	Unconfined compression	Equilibrium modulus (Young's modulus): 0.19 to 0.96 MPa

3. Evolution of mechanical testing protocols

Sample preparation and test set up

The sample extraction and preparation specifications have remained largely unchanged. Once the knee is dissected, cartilage strips are separated from the bones and 5 mm diameter samples are punched out for compression test and, 5mm by 1 mm sample are punched out for tensile test. The dimensions are measured using the optical thickness measurement system. All the tests are conducted at room temperature. Samples are taken from six locations in total. One compression and one tensile sample is taken out for a given location: medial and lateral femoral condyles, medial and lateral tibial plateau, patella and patellar groove.

All tests are conducted in a saline/phosphate buffered saline. There are specially designed chambers and clamps for appropriate tests and sample sizes. All tests are conducted on Mach-1 material testing system (Biomomentum Inc, Laval, Québec, Canada).

Mechanical tests (Confined and unconfined compression and Tensile test)

Initial test sets

- Initial proposed test protocol was; preload of 10g (to either establish contact with the sample in compression or determine initial length in tensile tests), preconditioning to 5% strain at 2Hz for 10 cycles, 5-10-15% strain at 100%/s strain rate with 45 min wait after every ramp.
- Data was acquired at 100Hz.
- Initial pilot samples and oks004 which was initially treated as a pilot specimen were tested with this protocol.
- For the given sample sizes, the system could not apply 100%/s strain rate accurately.

- After adjusting gains to try to improve the performance, it was apparent that a lower strain rate (preferably 20%/s) was consistently applied by the system. 20%/s was therefore chosen as the preferred strain rate going forward.

Beginning of repeatability tests

- During 'Initial length' and 'Find contact' sequences, force data was filtered.
- 100Hz sampling frequency was not adequate due to noise in force transducer output. It was decided that data will be sampled at 2kHz.
- Also 1g force was too low for initial contact establishment, 5g was selected. (A series of protocol evaluation sets were conducted to come up with various numbers for preload etc).
- With a series of repeatability tests, it was observed that confined compression and tensile tests were not repeatable. It was postulated, it may have been due to lower preload. It was increased to 10g.
- Target strain level for preconditioning was also increased to maximum strain of 15 % as to accommodate any fiber failure, mainly in tensile tests. These changes were reflected in all test protocols.
- Repeatability for confined compression and tensile tests was still not satisfactory. It was believed that maybe due to find contact being done prior to preconditioning but not after, immediately before the actual test loading. The tissue may settle in the clamp or the chamber after pre-conditioning which may shift the displacement at which force starts accumulating, i.e. zero force displacement. This may result in inadequate recruitment of tissue during loading and misleading comparison of force-displacement data.
- Another 'Find contact' / 'initial length' sequence was added after preconditioning.
- Note: The wait time after each ramp was ~3 min instead of specified 30 min.

- It was observed that the first test in the repeatability set for tensile samples in particular was stiffer than the following which were closer in behavior.
- Assuming preconditioning was not adequate, preconditioning was increased to 1000 cycles. This improved the results.
- During unconfined compression preconditioning, the prevent the sample from floating away, 5-15 % strain was used. This change then was reflected to all tests. (which was later changed to 10-15% as some samples still did not stay in place).
- Oks001 testing was started with protocols modified up to this point. Cartilage unconfined and confined tests were conducted.
- There appeared to be some buckling toward the beginning of the ramps in confined compression. This may be related to positioning of the indenter over the filter/sample. The sample also appeared to be stiffer in 5-10% strain region when compared to 10-15% strain region. This may point to some structural collapse at higher strains. This may also be attributed to the sample itself as the donor was 71 years old.
- Going forward, it was decided to conduct more repeatability tests using an older specimen as all the previous tests were conducted on a 40 year old specimen.
- 30 min hold time was used for all repeatability test thereafter. 3 mm wide indentor was made to cover larger area of filter during confined compression.
- A 48 yr old and 78 yr old specimens were ordered to conduct further extensive repeatability tests.
- A ramp load-unload stage was added before and after preconditioning to both assess failure if any before and after preconditioning and to characterize the elastic behavior of the tissue.
- It was decided that the indentor position in case of compression and clamp position in case of tensile tests would be kept 300 microns off of the contact position so as to capture the entire

loading of sample.

- Multiday tests with 24 hours between each test were conducted. Longer wait times between tests and use of PBS seemed to help recovery.
- Effect of multiple freeze thaw cycles was also assessed with unconfined compression. For the one set tested it appeared it took more than 7 cycles for the repeatability to deteriorate.
- 3 mm thick filter was decided upon to prevent buckling in confined compression.
- The tests from the 48 yr old specimen are more repeatable than those for the 78 yr old specimen. Confined compression tests are consistently more challenging to repeat.

Results for 48 yr old specimen:

1. Unconfined compression

oks00TR6-FMC-ACXX-01-01: Thickness = 2.3mm; 01 indentor positions:49.112,49.138

oks00TR6-FMC-ACXX-01-02: Thickness = 2.32 mm; 02 indentor positions: 39.85, 39.8595.

oks00TR6-FMC-ACXX-01-03: Thickness = 2.3 mm; 03 indentor positions: 39.794,39.8055

oks00TR6-FMC-ACXX-01-04: Thickness = 2.27 mm; 04 indentor positions: 34.5085,34.52

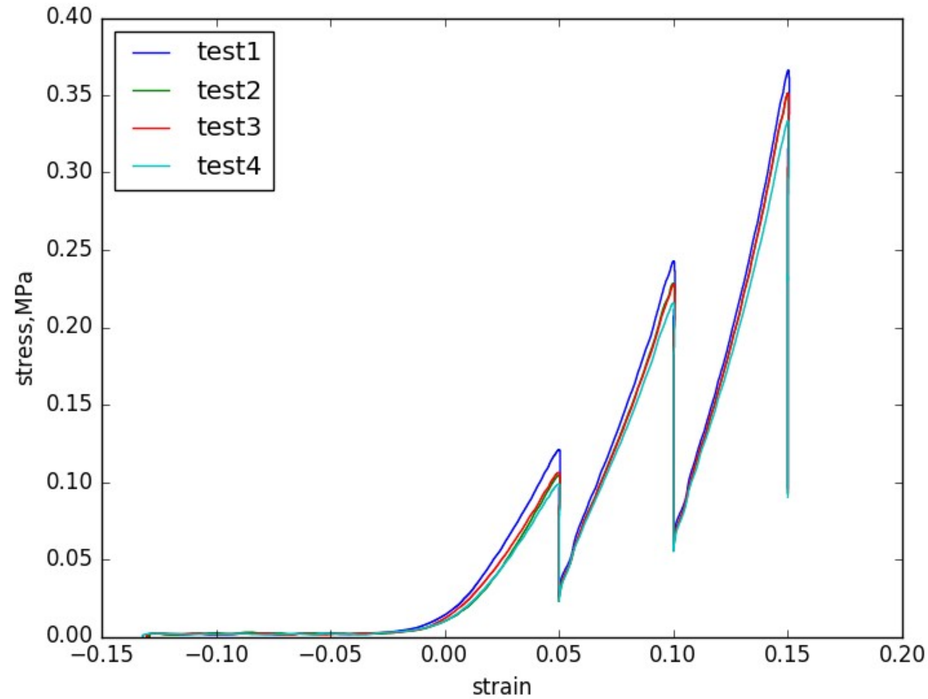

Figure 1: Unconfined compression repeatability behavior, 10g load location used as starting position

Peak force, g: 701.06 (23.14)

High strain instantaneous modulus, MPa: 2.64 (0.42)

High strain equilibrium modulus, MPa: 0.61 (0.037)

2. Confined compression

day 1: oks00TR6-FMC-MPXX-01-01: thickness: 1.79 mm, abandoned, used wrong value.

day 2: oks00TR6-FMC-MPXX-01-02: thickness: 1.8 mm, 02 indentor positions: 48.2485, 48.2585.

day 3: oks00TR6-FMC-MPXX-01-03: thickness: 1.82 mm, 03 indentor positions: 54.3935, 54.3995

day 4: oks00TR6-FMC-MPXX-01-04: thickness: 1.82 mm, 04 indentor positions: 48.1415, 48.147.

day 5: oks00TR6-FMC-MPXX-01-05: thickness: 1.818 mm, 05 indentor positions: 53.2195, 53.2185.

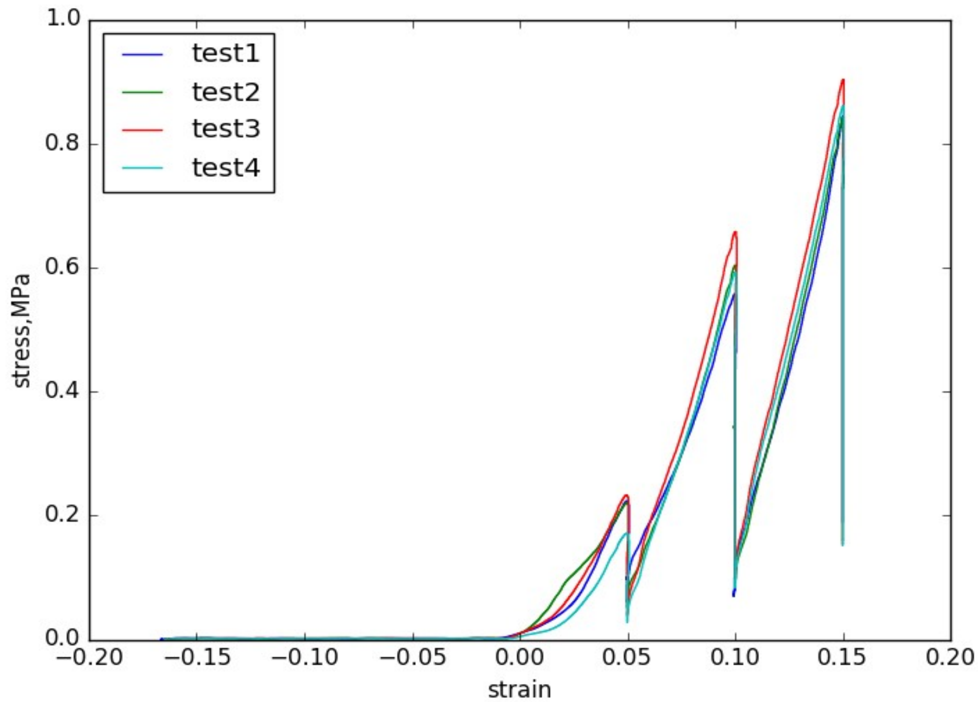

Figure 2: Confined compression repeatability behavior, 10g load location used as starting position

Peak force, g: 1715.47 (52.6)

High strain instantaneous modulus, MPa: 5.26(0.4)

High strain equilibrium modulus, MPa:1.315 (0.33)

3. Tensile test

oks00TR6-FMC-LPux-01-01: 5.6235,5.6760.

oks00TR6-FMC-LPux-01-02: 5.5735, 5.5980.

oks00TR6-FMC-LPux-01-03: 5.5895,5.6075

oks00TR6-FMC-LPux-01-04: 5.5995, 5.6255.

oks00TR6-FMC-LPux-01-05: 5.5785, 5.5995.

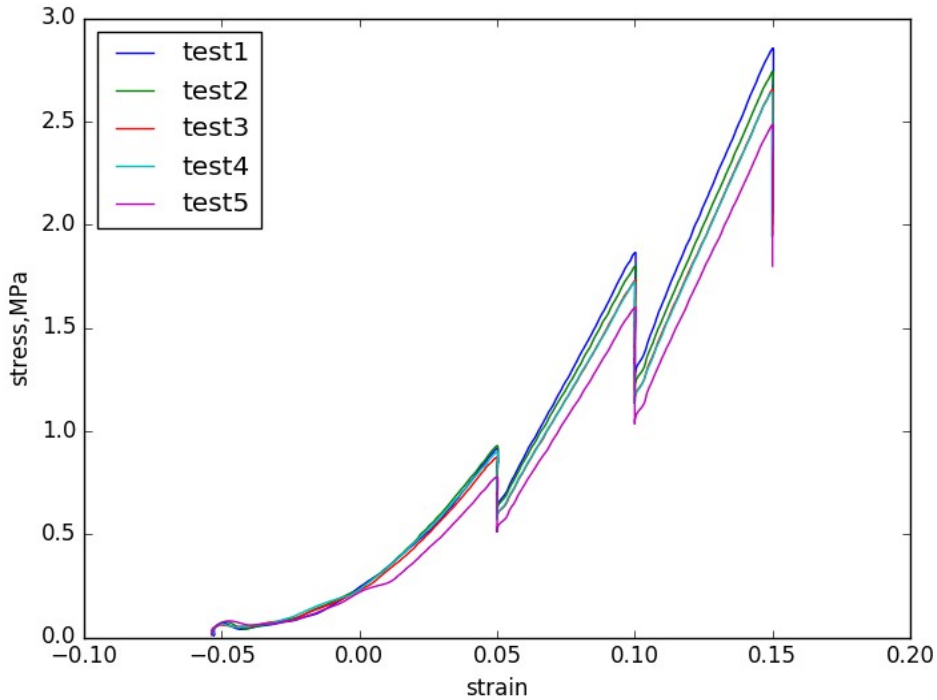

Figure 3: Tension repeatability behavior, 10g load location used as starting position

Peak force, g: 136.51 (6.99)

High strain instantaneous modulus, MPa: 18.57 (0.83)

High strain equilibrium modulus, MPa: 15.19 (0.26)

Results for 78 yr old specimen:

1. Unconfined compression

day 1: oks00TR5-FMC-ACXX-01-01: thickness: 2.36 mm; Positions: 57.06, 57.1055

day 2: oks00TR5-FMC-ACXX-01-02: thickness: 2.34 mm; Positions: 43.6945, 43.7125

day 3: oks00TR5-FMC-ACXX-01-03: thickness: 2.36 mm; Positions: 47.7325,47.7605.

day 4: oks00TR5-FMC-ACXX-01-04: thickness: 2.33 mm; Positions: 50.4035,50.4395.

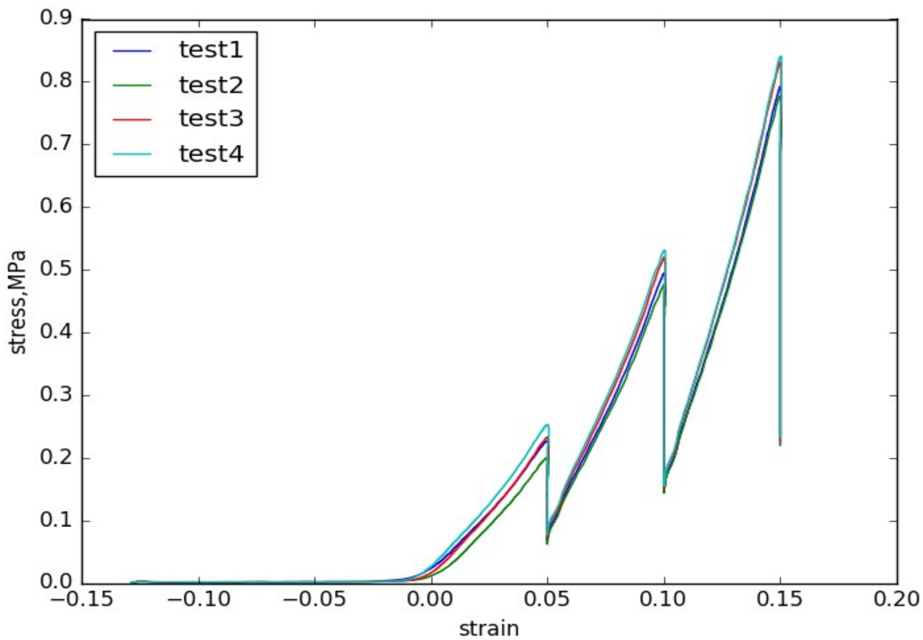

Figure 4: Unconfined compression repeatability behavior, 10g load location used as starting position

Peak force, g: 1620.37 (60.24)

High strain instantaneous modulus, MPa: 6.15 (0.18)

High strain equilibrium modulus, MPa: 1.42 (0.07)

2. Tensile test

Thickness: 0.408 mm

day 1: oks00TR5-FMC-LCuX-01-01: 6.6130, 6.6570.
mach 1 froze in second 'initial length-wait'. restarted software, waited 30 min before repeating. Used 1Hz camera data sampling freq (changed from 10Hz) going forward: 10 Hz camera rate used only for SR

day 2: oks00TR5-FMC-LCuX-01-02: 6.6940, 6.7065.

day 3: oks00TR5-FMC-LCuX-01-03: 6.5485, 6.5655.

day 4: oks00TR5-FMC-LCuX-01-04: 6.679, 6.6835

day 5: oks00TR5-FMC-LCuX-01-05: 6.6445, 6.6675.

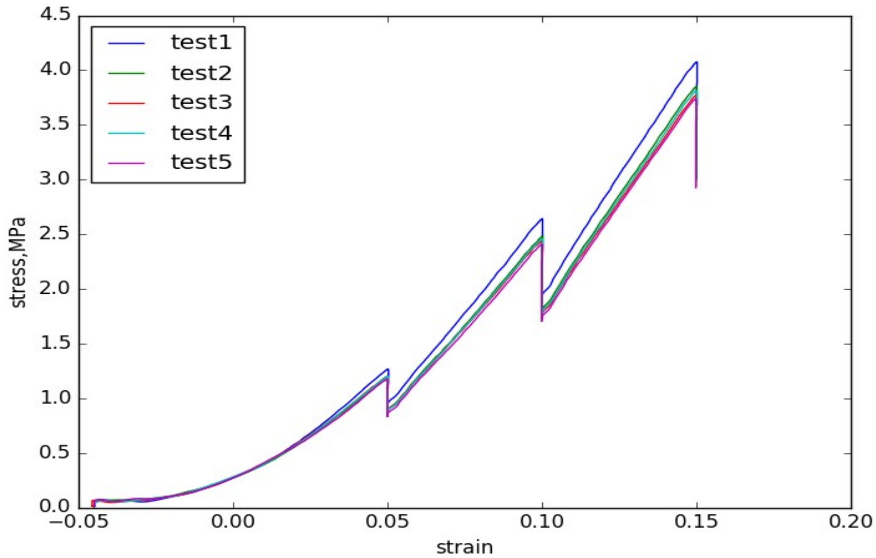

Figure 5: Tension repeatability behavior, 10g load location used as starting position

Peak force, g: 157.104 (5.4)

High strain instantaneous modulus, MPa: 26.434(0.93)

High strain equilibrium modulus, MPa: 23.136 (0.35)

Note: Confined compression repeatability tests did not have satisfactory results

4. References

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