Abstract—The United States patent, USP #6660829, pertains to the production of collagen-based products in the form of dispersions and macroporous structures using untreated raw fibrillar type I bovine corium as the starting material. The resulting dispersions have improved characteristics making them ideal for use in environmental applications as a settling aid, a filtration aid, a fractionation medium, an oil droplet stabilizer, a water purification aid, and a water siphoning aid. The dispersions may be further treated in accordance with the methods described in the patent to form macroporous structures suitable for environmental and biotechnological applications including use as a cell culturing substrate and non-biotechnological applications including use as an organic aerogel. Analysis of the admixtures produced by blending standard formulations of collagen dispersions with metal dust, inerts or metallic particles indicate that the collagen matrix may be used as a sacrificial scaffold. In this analysis, physical properties and microscopy were used to assess the quality of the dispersion. Statistical analyses were used to identify the potential for characterization using dynamic power law parameters, hysteresis, pore size, density, morphology, number and nature of crosslinks, and the possibility of connecting channels and flow-through. A modified power law formula and calculation technique is suggested for the characterization of the shear-stress-shear rate description of the rheology of the collagen dispersion. Experimental data and analyses are presented.

Keywords—nanofibrils, power law, shear stress, shear rate, kurtosis

I. INTRODUCTION

Collagen is a biodegradable polymeric fibrous protein found in all animals. In a series of steps, the collagen molecule assembles into a fiber that has the appearance of a rope. The material is insoluble in water, but can retain many times its own mass in water near its charged surface. This and the ability to unravel the fiber thus maximizing the surface area is the key physical property that leads to numerous environmental and biotechnological applications[1],[2],[3],[4],[5]. With regard to environmental applications, when added to sludge or any material with suspended solids, a collagen dispersion causes agglomeration, the formation of large flocs, and settling, all at a very rapid rate. The material has proven to be effective in the rapid agglomeration of fine solids in all types of sludge: industrial, water treatment, wastewater, inert suspensions, and kaolin.

It has been discovered that collagen dispersions may also be used in other environmental applications such as, as an aid to filtration, separation of pollutants (including metals and soluble organic molecules) from aqueous streams, selective fractionation of molecules, and oil droplet stabilization. Moreover, because treated collagen can hold hundreds of times its mass in water, its use in water purification (with minimal energy consumption) and in water siphoning has been discovered and quantified. All of these applications are based on the affinity of the activated surface of collagen, carrying positive charges, for the negative end of the polar water molecule.

Further processing of the dispersions yields products suitable for biotechnological applications[6],[7]. When the collagen dispersion is frozen and then freeze dried, the resulting material retains the overall dimensions of the original frozen material. However, over 99% of the volume is empty and the structure of the protein is a spongy organic aerogel with controllable pore size, good mechanical properties and a density of one thousandth of water. This solid material can be cross-linked to anchor or memorize its shape, pore size and morphology.

Covalent bonds, between adjacent collagen molecules, are formed during crosslinking; thus the resulting material will no longer disperse or retain water. When placed in water, the cross-linked collagen sinks because the specific gravity is slightly higher than that of water. During the process of crosslinking, the material that is produced is also sterile. This material has enormous potential in biotechnology especially in the area of cell culture. Some of the cell culture applications include substrates for: a) achieving high cell density in bioreactors leading to increased productivity and reduced reactor sizes; b) hosting unusual and hard-to-culture cells that are used for a variety of applications including biosensors; c) organ and tissue technology that have medical implications (examples are organ regrowth, skin replacement, coating of prostheses and implants, etc.); d) coating of cell culture devices such as roller bottles or glass beads; e) collagen membranes for cell culture and biomolecule delivery; and f) controlled release of pharmaceuticals. In non-biotechnology applications the freeze dried, cross-linked collagen matrix can serve as an organic aerogel. Other possible uses for this material include encapsulation of a wide variety of organisms, enzymes and synthetic material.
II. MATERIALS AND METHODS – POWER LAW PARAMETER

A. Method to Develop Collagen Nanofibrils
1. Weighed approximately 1kg of the Raw Bovine Dermis collagen sheets
2. Placed into ball mill with zirconia beads
3. Filled mill with deionized water until it covered the collagen and beads
4. Closed, sealed, and let run for approximately 2 days
5. Collected milled collagen and placed equally into 4 centrifuge bottles
6. Ran centrifuge at 5°C for 15 minutes to separate the water from the collagen nanofibrils
7. Top liquid was discarded and centrifuge bottles were filled with clean DI water
8. Ran centrifuge twice more until top liquid looked clean
9. Collagen paste was ready to use and stored in the refrigerator

B. Method of Formulating 1% Collagen Dispersion
1. Added acetic acid and deionized water by weight to pre-weighed amount of collagen nanofibrils produced above
2. Blended for approximately 5 minutes or until dispersion became thicker and homogenous
3. Stored in refrigerator for later use

C. Method of Experimental Tests
1. Previously made 1% and 2% collagen dispersions were taken out of refrigerator and left out for an hour
2. These samples were analyzed by a Viscometer that collected the viscosity, shear stress, shear rate, and pressure
3. Each day, four replicated and two treatments (increase and decrease of shear rate) were performed using Viscometer
4. Viscosity, shear stress, shear rate and pressure data was monitored and recorded
5. These tests ran for a total of 11 days until the shear stress became steady
6. Data was collected and analyzed by excel

D. Summary of the Dispersion Process
Raw collagen from a variety of sources is the starting material in the manufacture and modification of collagen nanofibrils. The raw material has the appearance of white ground protein as shown in Fig. 1.

It has been discovered that the above described existing collagen-based applications are enhanced, and novel applications possible, using raw fibrillar type I bovine corium as the starting material. Corium is the dermis layer of the hide and is rich in collagen-based connective tissue. While corium has been indicated as a preferred source of collagen for at least some applications, the applicant has discovered that use of a heterogeneous solution of corium as the starting material, as opposed to purified collagen derived from corium, produces superior end-products including new applications and results not heretofore observed.

Previously, corium was pre-treated to remove fats, triglycerides, and other soluble compounds. The resulting raw collagen was then conventionally dried and milled in a knife mill. In the present invention, a dilute solution of the corium itself is milled in a ball mill containing zirconia media for one to two weeks. The pretreatment steps are avoided. Once milling is completed, the resulting material is strained, washed, and then subjected to low temperature centrifugation and the supernatant decanted. This process is repeated several times until no fats or other soluble materials appear in the upper phase and the supernatant is clear. The lower phase containing collagen is then blended in a solution containing an organic acid to form a dispersion and allowed to thicken. The resulting dispersion has improved physical properties and results in enhanced performance when used in various environmental applications.

The above dispersion may be further processed to form physically improved collagen macroporous structures or substrates capable of utilization in various biotechnological applications. During the blending stage any material for encapsulation or controlled release is added.
There has thus been outlined, rather broadly, some important features of the invention in order that the detailed description thereof that follows may be better understood, and in order that the present contribution to the art may be better appreciated. There are, of course, additional features of the invention that will be described hereinafter and which will form the subject matter of the claims appended hereto. In this respect, before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and to the arrangements of the components set forth in the following description or illustrated in the drawings.

This invention is capable of other embodiments as presented and of being practiced and carried out in various ways, biomedical and environmental. Also, it is to be understood that the phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting. As such, those scientists and engineers working in protein technology will appreciate that the conception, upon which this research is based, may readily be utilized as a basis for the designing of other structures, methods and systems for carrying out the several purposes of the present invention[9],[10],[11],[12].

### III. DATA: ANALYSIS OF RESULTS

#### A. ANOVA TEST – F Distribution and t-Distribution

| Day 2: Data From 3.57% Collagen Paste |
|-------------------------------------|
| Parameter(a) | Parameter(b) | Directions |
|-------------|-------------|------------|
| Trial 1     | 7.2218      | 0.1978     | Increase   |
|             | 4.8652      | 0.2750     | Decrease   |
| Trial 2     | 5.1141      | 0.2389     | Increase   |
|             | 5.0947      | 0.2360     | Decrease   |
| Trial 3     | 5.1732      | 0.2321     | Increase   |
|             | 5.0405      | 0.2357     | Decrease   |
| Trial 4     | 5.1732      | 0.2244     | Increase   |
|             | 5.0385      | 0.2318     | Decrease   |
| average a   | 5.3401      |             |            |
| std. dev. a | 0.7666      |             |            |
| average b   | 0.2340      |             |            |
| std. dev. b | 0.0211      |             |            |

Table 1 shows the average value of parameter(a) and Parameter(b) in different treatments (increase/ decrease the shear rate) for day 2, and also the average value and standard deviation of parameter (a) and parameter (b) for all four trials.

| Day 2: t – Distribution |
|-------------------------|
| Testing of Means       | Observations |
| Do increase first       | H0: b=0.22   | t0  | 0.3666 |
|                         | H1: b ≠ 0.22 | absolute(t0) | 0.3666 |
|                         | t(0.05,3)    | 3.1824  | |
| If t > t1, reject      | Fail to reject, therefore, b=0.22 |
| Do decrease second      | H0: b=0.24   | t0  | 0.4548 |
|                         | H1: b ≠ 0.24 | absolute(t0) | 0.4548 |
|                         | t(0.05,3)    | 3.1824  | |
| If t ≤ t1, reject      | Fail to reject, therefore, b=0.24 |

Table 2 is the t-Distribution for day 2, from the t-distribution, one can conclude that the increase and decrease treatments don’t matter for the value of parameter(b), where increase means increase the shear rate, and decrease means decrease shear rate to return to original shear rate. The purpose of the treatment is to test the hysteresis.
Table 3 is the data analysis for One-Way ANOVA for the error and total Sum of Square for day 2.

### TABLE IV. DAY 2 DATA ANALYSIS FOR TREATMENT

|               | ss treatments | ss errors | ss Total | F(0.05,1,6) | H0: Treatments( increase, decrease) don't matter | H1: Treatments matter |
|---------------|---------------|-----------|----------|-------------|-------------------------------------------------|-----------------------|
| Day 2: ANOVA  |               |           |          |             |                                                 |                       |
| Between treatments | 0.00910      | 0.002213  | 0.003123 | 5.9874      | fail to reject, therefore, treatments don't matter | treatments matter      |
| Mean square    | 1             | 6         | 7        |             |                                                 |                       |
| F0             | 2.4654        |           |          |             |                                                 |                       |

Table 7 is the data analysis for One-Way ANOVA for the error and total Sum of Squares for Day 6.

### TABLE VIII. DAY 6 DATA ANALYSIS FOR TREATMENT

|               | ss treatments | ss errors | ss Total | F(0.05,1,6) | H0: Treatments( increase, decrease) don't matter | H1: Treatments matter |
|---------------|---------------|-----------|----------|-------------|-------------------------------------------------|-----------------------|
| Day 6: ANOVA  |               |           |          |             |                                                 |                       |
| Between treatments | 0.000425     | 0.000863 | 0.001288 | 5.9874      | fail to reject, therefore, treatments don’t matter | treatments don’t matter |
| Mean square    | 0.000144      |           |          |             |                                                 |                       |
| F0             | 2.9522        |           |          |             |                                                 |                       |

Table 8 is the Test on Means of Normal Distribution-Variance Known Analysis for Day 6.

### TABLE IX. DAY 6 DATA FROM 3.57% COLLAGEN PASTE

| Parameter(a) | Parameter(b) | Directions |
|--------------|--------------|------------|
| Trial 1      | 7.3082       | 0.2023     | increase  |
| Trial 2      | 5.7185       | 0.2477     | decrease  |
| Trial 3      | 5.8183       | 0.2385     | increase  |
| Trial 4      | 5.6019       | 0.2411     | decrease  |
| average a    | 5.8289       |            |           |
| std. dev. a  | 0.6101       |            |           |
| average b    | 0.2334       |            |           |
| std. dev. b  | 0.0136       |            |           |

Table 9 shows the average value of parameter(a) and parameter(b) in Different treatments (increase/ decrease shear rate) for day 11, and the average value and standard deviation of parameter (a) and parameter (b) for all four trials.

### TABLE X. DATA ANALYSIS FROM DAY 11 RESULTS

|               | ss treatments | ss errors | ss Total | F(0.05,1,6) | H0: Treatments( increase, decrease) don't matter | H1: Treatments matter |
|---------------|---------------|-----------|----------|-------------|-------------------------------------------------|-----------------------|
| Day 11: ANOVA |               |           |          |             |                                                 |                       |
| Between treatments | 0.000425     | 0.000863 | 0.001288 | 5.9874      | fail to reject, therefore, treatments don’t matter | treatments don’t matter |
| Mean square    | 0.000144      |           |          |             |                                                 |                       |
| F0             | 2.9522        |           |          |             |                                                 |                       |

Table 6 is the t-Distribution for day 6, from the t-distribution, one can conclude that treatments(increase and decrease shear rate) don’t matter for the value of parameter(b).

### TABLE VII. DAY 6 SS ANALYSIS

| Trial   | Increase | Decrease | Average | std. dev. | Observations |
|---------|----------|----------|---------|-----------|--------------|
| 1       | 0.2023   | 0.2477   |         |           |              |
| 2       | 0.2385   | 0.2411   |         |           |              |
| 3       | 0.2321   | 0.2375   |         |           |              |
| 4       | 0.2316   | 0.2365   |         |           |              |
| Average | 0.2261   | 0.2407   | 0.2334  | 0.0051    |              |

Table 10 is the t-Distribution for day 11, from the t-Distribution, one can conclude that the increase and decrease treatments don’t matter for the value of parameter(b).
Table 11 is the data analysis for One-Way ANOVA for the error and total Sum of Square for day 11.

Table 12 is the Test on Means of Normal Distribution-Kurtosis and Skewness for 1% collagen dispersion of parameter(a) and parameter(b) in 11 days.

Table 13 is the average data of 1% collagen dispersion made from 3.57% collagen paste for parameter(a) and parameter(b) in 11 days.

Table 14 is the analysis for average parameter (a), the kurtosis value of 6.3142 is greater than 3. And skewness 2.3915 is greater than 1. Both data are higher than the normal kurtosis and skewness.

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**Table XI.** DAY 11 SS ANALYSIS

| Day 11: SS Analysis | Increase | Decrease |
|---------------------|----------|----------|
| Trial 1             | 0.2036   | 0.2569   |
| Trial 2             | 0.2530   | 0.2466   |
| Trial 3             | 0.2434   | 0.2391   |
| Average             | 0.2339   | 0.2451   |
| std. dev.           | 0.0214   | 0.0088   |
| Observations        | 0.9355   | 0.9802   |
| Total               | 0.9355   | 0.9802   |
| Average             | 0.2339   | 0.2451   |
| ss treatments       | 0.000250 |          |
| Trial 1             | 0.000917 | 0.000140 |
| Trial 2             | 0.000366 | 0.000002 |
| Trial 3             | 0.000091 | 0.000035 |
| Trial 4             | 0.000003 | 0.000056 |
| Total               | 0.001376 | 0.000234 |
| ss error            | 0.001609 |          |
| ss Total            | 0.001859 |          |

Table 11 is the data analysis for One-Way ANOVA for the error and total Sum of Square for day 11.

**Table XII.** DAY 11 DATA ANALYSIS FOR TREATMENT

| Day 11: ANOVA |
|---------------|
| Between treatments | ss     | DOF | Mean square | F0  |
| ss treatments    | 0.000250 | 1   | 0.000250    | 0.9311 |
| ss errors        | 0.001609 | 6   | 0.000268    |     |
| ss Total         | 0.001859 | 7   |             |     |
| F(0.05,1,6)      | 5.9874  |     |             |     |
| H0:              | Treatments( increase, decrease) don't matter |
| H1:              | treatments matter |
| If F>Fo, reject H0 | fail to reject, treatment don't matter |

Table 12 is the Test on Means of Normal Distribution-Kurtosis and Skewness for 1% collagen dispersion of parameter(a) and parameter(b) in 11 days.

**Table XIII.** AVERAGE DATA OF POWER LAW PARAMETER FOR 1% COLLAGEN DISPERSION IN 11 DAYS

| 1% collagen from 3.57% paste |
|-------------------------------|
| Day                  | average parameter a | Average parameter b |
| 1                    | 4.97               | 0.197               |
| 2                    | 5.34               | 0.23                |
| 3                    | 5.22               | 0.22                |
| 4                    | 4.92               | 0.23                |
| 5                    | 6.53               | 0.23                |
| 6                    | 5.83               | 0.23                |
| 7                    | 5.45               | 0.24                |
| 8                    | 8.63               | 0.18                |
| 9                    | 5.68               | 0.25                |
| 10                   | 5.5                | 0.24                |
| 11                   | 5.38               | 0.24                |

Table 13 is the average data of 1% collagen dispersion made from 3.57% collagen paste for parameter(a) and parameter(b) in 11 days.

**Table XIV.** KURTOSIS AND SKEWNESS DATA OF PARAMETER A

| Analysis for Average parameter(a) |
|-----------------------------------|
| Mean                              | 5.7682               |
| Standard Error                    | 0.3155               |
| Median                            | 5.4500               |
| Mode                              | N/A                  |
| std. dev. (Standard Deviation)    | 1.0462               |
| Sample Variance                   | 1.0946               |
| Kurtosis                          | 6.3142               |
| Skewness                          | 2.3915               |
| Range                             | 3.7100               |
| Minimum                           | 4.9200               |
| Maximum                           | 8.6300               |
| Sum                               | 63.4500              |
| Count                             | 11.0000              |
| Confidence Level (95.0%)          | 0.7029               |
| UL (Upper Limit)                  | 6.4711               |
| LL (Lower Limit)                  | 5.0653               |

Table 14 is the analysis for average parameter (a), the kurtosis value of 6.3142 is greater than 3. And skewness 2.3915 is greater than 1. Both data are higher than the normal kurtosis and skewness.

**Table XV.** KURTOSIS AND SKEWNESS DATA OF PARAMETER B

| Analysis for Average parameter b) |
|-----------------------------------|
| Mean                              | 0.2361               |
| Standard Error                    | 0.0062               |
| Median                            | 0.2300               |
| Mode                              | 0.2300               |
| std. dev. (Standard Deviation)    | 0.0305               |
| Sample Variance                   | 0.0004               |
| Kurtosis                          | 1.6685               |
| Skewness                          | -1.4234              |
| Range                             | 0.0700               |
| Minimum                           | 0.1800               |
| Maximum                           | 0.2500               |
| Sum                               | 2.4870               |
| Count                             | 11.0000              |
| Confidence Level (95.0%)          | 0.0138               |
| UL (Upper Limit)                  | 0.2399               |
| LL (Lower Limit)                  | 0.2123               |
Table 15 is the analysis for average parameter (b), the kurtosis value of 1.6685 is less than 3. And skewness -1.4204 is out of the range between -1 and 1. Kurtosis value is in the range of the normal kurtosis, but skewness value is out of the normal range.

C. Goal Seek Application for R²

Goal seek is computed to further analyze the data to pick a power that is as close to 0.9999 as possible. In Table 16, these data shows the goal seek application for picked power law equation to reach the maximum R² value. The shear rate and shear stress data came from the 1st trial decrease, R² =0.9386 originally of 1% collagen dispersion made from 3.57% collagen at day 2. Following is the Power Law Equation:

\[ SS= a + b \cdot SR^c \]  

SS= Shear stress, Pa  
SR= Shear rate, 1/s  
a,b,c=modified power law parameter for non-Newtonian fluids[15].

| Day 2: Applying goal seek to get R² | Shear rate, 1/s | Shear Stress, Pa | Shear rate⁴ |
|-----------------------------------|----------------|-----------------|-------------|
| 0.418                             | 4.1089         | 0.4648          |
| 0.522                             | 4.2125         | 0.5649          |
| 0.836                             | 4.7401         | 0.8544          |
| 1.045                             | 4.9533         | 1.0394          |
| 2.09                              | 5.7893         | 1.9108          |
| 4.18                              | 6.8134         | 3.5129          |
| 10.45                             | 7.6076         | 7.8564          |
| 12.51                             | 9.2199         | 9.2016          |
| 20.94                             | 10.7003        | 14.4672         |
| 41.82                             | 17.4808        | 26.5627         |

Goal seek approach
slope 0.4854  
intercept 4.3378  
pick power in power law equation 0.8784

In Table 17, these data shows the goal seek application for picked power in power law equation to reach the maximum R², shear rate and shear stress data came from 1st trial increase, R² =0.9932 originally of 1% collagen dispersion made from 3.57% collagen at day 6.

| Day 6: Applying goal seek to get R² | Shear rate, 1/s | Shear Stress | Shear rate⁴ |
|-----------------------------------|----------------|--------------|-------------|
| 0.418                             | 5.9356         | 0.9395       |
| 0.522                             | 6.2640         | 0.9546       |
| 0.836                             | 7.0642         | 0.9873       |
| 1.045                             | 7.4300         | 1.0032       |
| 2.09                              | 8.7362         | 1.0541       |
| 4.18                              | 10.2410        | 1.1077       |
| 10.45                             | 12.0175        | 1.1827       |
| 12.51                             | 12.0096        | 1.1980       |
| 20.94                             | 13.4435        | 1.2429       |
| 41.82                             | 15.0134        | 1.3059       |

Goal seek approach
slope 24.7349  
intercept -17.3331  
pick power in power law equation 0.0715

In Table 18, these data shows the goal seek application for picked power in power law equation to reach the maximum R², shear rate and shear stress data came from 1st trial increase, R² =0.9962 originally of 1% collagen dispersion made from 3.57% collagen at day 11.

| Day 11: Applying goal seek to get R² | Shear rate, 1/s | Shear Stress | Shear rate⁴ |
|------------------------------------|----------------|--------------|-------------|
| 0.418                              | 5.4758         | 0.9245       |
| 0.522                              | 5.7420         | 0.9432       |
| 0.836                              | 6.4288         | 0.9840       |
| 1.045                              | 6.7403         | 1.0040       |
| 2.09                               | 7.9002         | 1.0686       |
| 4.18                               | 9.1124         | 1.1373       |
| 10.45                              | 11.0770        | 1.2351       |
| 12.51                              | 11.0088        | 1.2552       |
| 20.94                              | 12.4384        | 1.3148       |
| 41.82                              | 13.7588        | 1.3992       |

Goal seek approach
slope 17.6879  
intercept -10.9590  
pick power in power law equation 0.0900

In Table 19, these data shows the goal seek application for picked power in power law equation to reach the maximum R², shear rate and shear stress data came from 1st trial increase, R² =0.9962 originally of 1% collagen dispersion made from 3.57% collagen at day 11.
IV. APPENDIX 1: POWER LAW FORMULA

\[ \tau = \mu \left( \frac{\partial \vec{v}}{\partial r} \right) \] Newton's Law

\[ \tau = \left( \frac{\partial \vec{v}}{\partial r} \right) \] Power Law Fluid; \( b > 1 \) dilatant; \( b < 1 \) pseudoplastic

\[ \frac{\Delta P}{L} = \frac{1}{\mu} \left( \frac{\partial \vec{v}}{\partial r} \right) \] Equation of Motion for Newtonian Fluid in a Pipe

\[ \frac{\Delta P}{L} = \frac{1}{\mu} \left( \frac{\partial \vec{v}}{\partial r} \right) \] Equation of Motion for Power Law Fluid in a Pipe

\[ \frac{\Delta P}{L} = \frac{1}{\mu} \left( \frac{\partial \vec{v}}{\partial r} \right) \] Equation of Motion for Collagen Dispersions

where: \( \tau \) is shear stress for flow in the z direction (Axial) with stress at a plane at r, \( \mu \) is the proportionality constant in Newton’s Law, typically called viscosity, \( \frac{\Delta P}{L} \) is the pressure drop in Pa per length of pipeline in m, a, b, c, τ₀ are the modified power law parameters for non-Newtonian fluids

SS = \( \tau = \alpha \cdot \text{SR}^c \) regressed as

SS = \( \tau \) vs. \( \text{SR}^c \)

At Day 6, 1% of collagen dispersion was taken out from the refrigerator and then left for an hour. Then the 1% dispersion was analyzed by viscometer. Followed by monitoring and recording Viscosity, shear stress, Shear rate and pressure data in both increasing and decreasing shear rate directions. And then excel was used to analyze the data collected.

V. CONCLUSION

From all the data collected, power law Intercept is not zero for log equation. After series of data analysis, ln (shear stress) vs. ln (shear rate) equation are linear with non-zero intercept. Therefore, the power law formula was then rewrite by Taking power law until it is Close to before by using Goal seek to get \( R^2 \) as close to 0.9999. In conclusion, we discovered that from day 1 to day 11, it follows the Power law. As the time increased, parameter (a) kept increasing. Parameter (b) kept constant. the shear stress increased as the time increased until day 11, shear stress kept constant.

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