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Full Length Research Paper

Preliminary investigation of tensile properties of welded type I collagen to be used as surgical sealant

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Type I collagen has been identified as the most abundant protein in the human body, and it has been used extensively in the healthcare industry as a biomaterial for the purposes of soft tissue repair, tissue engineering and many other areas. The aim of this study was to research the mechanical tensile strength of type I collagen when welded together for possible use as a sealant biomaterial for surgical closure. Sheets of type I collagen from calf skin were prepared using collagen granules and a 1.6 M acetic solution. The sheets were cut into smaller pieces and welded together at different widths using a magnetic heat plate. Mechanical tensile properties were subsequently determined using a tensiometer. A set of six parameters were selected as the variables of choice for the purpose of analyzing the mechanical properties, namely, the tensile stress and tensile strain at the point of failure, the maximum load that was applied to the collagen sample and the physical dimensions of each of the collagen samples. It was found that only the tensile strain at failure point is significantly different ($P < 0.01$) between the different groups of collagen samples. However, a conclusive decision could not be made based on this finding alone, and further investigations are warranted.

Key words: Collagen, biomaterials, sealant.

INTRODUCTION

Collagen is one of the most abundant and ubiquitous proteins in the human body. It can be found in every major tissue in the human body that requires strength and flexibility; such as tendon, skin, bone and fascia. To date, 19 types of collagen that have been identified, with type I collagen being the most abundant and a major component of bone, skin, ligament and tendon (Park and Bronzino, 2003; Tiyek et al., 2011). The collagen molecule is made up of a triple helix formed by three polypeptides chains. The triple helix consists of a sequence of $(-G-X-Y)_n$, where G is glycine and X and Y is often proline or hydroxyproline amino acids (Kim et al., 2000; Park and Bronzino, 2003). Type I collagen has a molecular weight of 280,000 daltons, and is comprised of three polypeptide chains that are organized into a central helix configuration (Park and Bronzino, 2003; Tiyek et al., 2011). Two of the polypeptide chains are identical, with 1,056 acid amino residues on one chain and 1029 on the other. The collagen molecule is stabilized by hydrogen bonds, in which the majority is associated with hydroxyproline. The type I collagen structure shows a characteristic 67 nm periodicity that comes from quarter-staggered assemblies of 4 to 5 molecules into microfibrils (Kim et al., 2000; Tiyek et al., 2011). Figure 1 shows the molecular/fibrillar configuration of type I collagen.

Collagen has been used extensively in the healthcare industry as biomaterials in soft tissue repair, tissue engineering and many other areas. Pachence (1996) listed the reasons why collagen was chosen as the biomaterials of choice for many medical applications:

1. Know-how for obtaining large quantities of medical grade collagen is well developed;
2. There are a number of established collagen products, some of which are well known;
3. Collagen has a good safety profile as biomaterial;
4. Collagen can be produced in forms that are easily used

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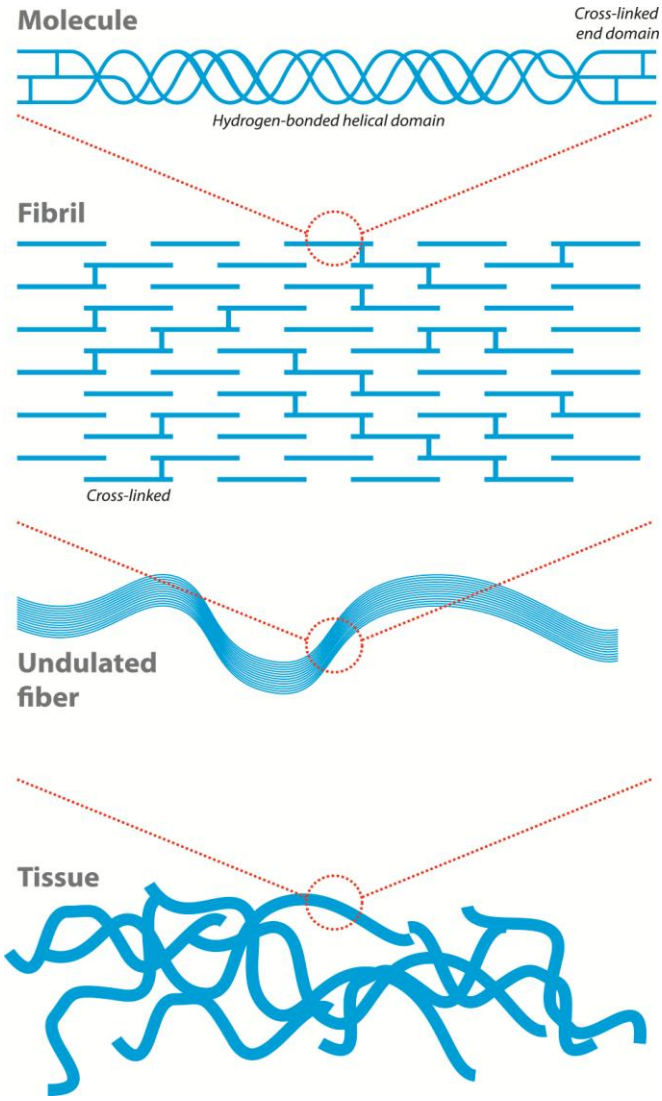

Figure 1. The molecular/fibrillar configuration of type I collagen (Kim et al., 2000).

in minimally invasive procedures;
5. The understanding of collagen's role in wound healing, metabolism and catabolism, and the interaction between cells and collagen has been greatly improved.

Many studies have been conducted with regards to mechanical properties of collagen in different applications (Pachence, 1996; Chen et al., 1998; Rocha et al., 2002; Sturgis et al., 2002; Wright and Humphrey, 2002; Feng et al., 2003, 2006; Chandran and Barocas, 2004; Lu et al., 2004; Cheung et al., 2007). One study investigated the mechanical properties of contracted collagen gels as a scaffold in tissue engineering. In this study, type I collagen is used to determine the mechanical strength of large collagen gels populated with human fibroblast. The researchers used uniaxial tensile, strength and rheological tests in the study and the results revealed that the human fibroblasts significantly contacted collagen gels in order to achieve certain mechanical strength. This finding shows that it is possible for the contacted collagen gels to act as a scaffold for tissue engineering. In a separate study, the tensile properties of three-dimensional type I collagen extracellular matrices were investigated. Understanding the mechanical properties of extracellular matrix (ECM) scaffolds for tissue engineering is important as a means of ensuring that the ECM would be able to achieve its intended function in the natural behavior of the cells and its surroundings.

Another possible use for collagen is for the purpose of surgical closure, whereby it is currently being considered as a possible alternative to sealant material. Although, the gold standard for surgical closure remains the suture and the staple; there are many instances whereby the suture is ineffective and/or interferes with the healing process of the wounded site. This is where sealant material is advantageous. Sealant material includes fibrin glues, albumin solders, and synthetic polymeric materials (e.g. cyanoacrilates and polyethylene glycol) and the wound closures that use these surgical adhesives have been found to be effective in certain cases where conventional use of sutures or staples have failed and/or problematic side-effects have occurred.

Fibrin sealants have become one of the most popular types of sealants in use today, primarily due to the contributory role they play in the final stages of the natural wound healing process. Polymerisation of fibrin monomers, forms the physiological fibrin clot, and this is followed by tissue adhesion, cellular proliferation and infiltration, and finally, collagen fibers proliferation. The end stage of the healing is reached with the formation of collagen-rich granulation tissue. However, despite their popularity, serious complications can arise with the use of fibrin sealants, and this is why the search continues for alternative materials that can provide the same hemostatic and tissue sealing properties.

One of the materials that are being proposed is collagen, due to the fact that it is the most abundant protein in the human body. In order to study its usefulness as a sealant, it is useful to investigate its mechanical properties. The main objective of this study is therefore to investigate the tensile properties of welded collagen, and to provide qualitative analysis as to whether its mechanical strength is sufficient for it to act as a surgical sealant. The mechanical characteristics that will be investigated include the tensile stress, strain and strength.

MATERIALS AND METHODS

Collagen sheet preparation

Type I collagen (Sigma-Aldrich Co., St Louis, Missouri, USA) from calf skin was used in this study. The amount of concentration needed in order to produce a collagen solution that does not crystallize has been found to be 16 mg/ml. To prepare a solution of

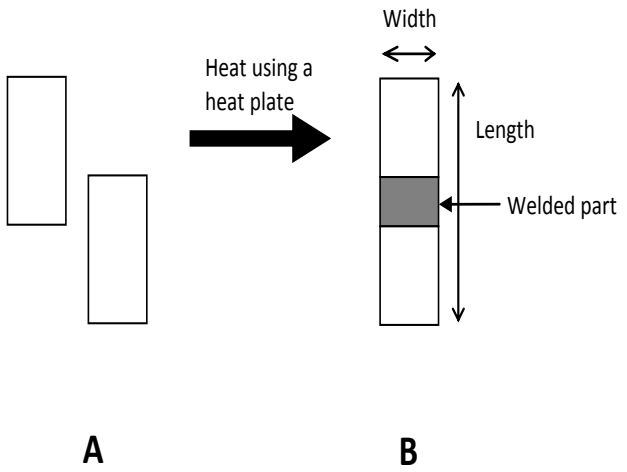

Figure 2. Schematic diagram of (A) the alignment of the collagen pieces prior to heating and (B) the measurement that is needed before proceeding with tensile testing. The shaded area shows the welded part.

this concentration using 0.1 M acetic acid, the correct amount of acetic acid required (in ml) was determined by dividing the amount of collagen (in mg) with the amount of desired concentration (16 mg/ml). This mixture was stirred manually and was left overnight before being used for the preparation of the collagen sheets the following day.

A plastic slab of approximately 5 × 20 cm was used as a base upon which the preparation of the collagen sheets could take place. Five 2 × 2 cm squares were drawn on the slab as a guide to apply the collagen solution. A sufficient amount of the collagen solution was carefully poured onto the slab to fill up the drawn squares, ensuring that it did not exceed the area marked by each of the squares. The slab was then left in the fume cupboard at room temperature for at least 24 h in order to ensure the collagen solution had totally dried and the collagen sheets were formed.

Collagen welding

The prepared collagen sheets needed to be cut into smaller pieces to be welded and used in the subsequent tensile tests. In order to do this, each of the collagen sheets was carefully peeled from the plastic slab and placed on a petri dish. Using a scalpel, each collagen sheet was then cut into rectangular shapes, maintaining a width of approximately 4 mm. This was necessary in order to ensure that the ends of the collagen sheet pieces were sufficiently wide to be held by the grip of the tensiometer.

The welding process was initiated by overlying two pieces of cut collagen sheets on one of the edges (Figure 2). The two pieces were carefully transferred onto the heat plate using tweezers. Aluminum foil the size of approximately 10 × 10 cm was laid onto the plate prior to the transfer of the collagen pieces in order to avoid possible contamination of the collagen pieces by the surface of the plate. The pieces were placed in position and a metal block covered in polytetrafluoroethylene (PTFE) material was used to ensure proper adhesion of the collagen pieces. The temperature of the heat plate was maintained at approximately 700°C, and this was monitored using a thermocouple in order to ensure uniform heating of the collagen sheets. The pieces were left on the plate for 30 s, before they were removed and their dimensions measured. The width and the length of the welded pieces and the thickness of the welded area (depicted by the shaded area in Figure 2) were measured for the purpose of later tensile testing, when the parameters were entered into the tensile testing software prior to the commencement of the test. The procedure was then repeated for each pair of the collagen pieces that had been previously cut.

Tensile testing

The tensile tests were conducted using an Instron tensiometer (Instron Corp., Canton, Massachusetts, USA). This machine is equipped with a 50 N load cell and tests were conducted at the speed of 20 mm/min. The unit was connected to a computer, in which proprietary software was installed for the purposes of data calculation and measurement. The dimensions of the welded collagen pieces that measured post collagen welding were entered into the software prior to the commencement of the tensile test.

The welded collagen pieces were then tested one at a time. Each welded collagen piece was placed between the two grips of the tensiometer, ensuring that the piece was properly aligned in order to allow even and equal stretching to take place between the two ends. The force was then applied incrementally according to the speed that was previously selected, and the value was measured by the load cell. The displacement of the collagen sample was simultaneously measured by the displacement sensor. The test was terminated once the collagen piece broke in two, and the behavior of the failure was also observed and documented. The tensile test was then repeated for each of the welded collagen pieces, and the collected tensile test data was saved for later analysis.

Statistical analysis

The data for the tensile stress and strain at the point of failure, and the maximal load sustained by each of the collagen pieces was analyzed using the analysis of variance (ANOVA). This was performed as a means of determining whether the mean values of the aforementioned parameters were equal, and whether the differences that may exist were significant.

RESULTS

As mentioned in the Introduction, this study was performed on three different sets of collagen over two different experimental occasions. Groups 1 and 2 consisted of welded collagen samples, while group 3 consisted of single unwelded collagen samples. Each of the sets consisted of seven samples of collagen pieces, and all seven pieces were prepared and tested at the same time in one experimental run. The purpose of this was to ensure that every collagen sample in the same group was treated and prepared in the same experimental conditions. Although, the software used in the tensile testing was able to produce numerous mechanical datasets for each of the tested sample, only six of these were selected to be included in the study. These results include the tensile stress and tensile strain at the point of failure, the maximum load that was applied to the collagen sample and the dimensions of each of the collagen sample (thickness, width and area). The mean, the standard deviation and the range for each of these datasets is also included for each of the collagen sample groups tested.

Table 1. Group 1 (welded collagen) tensile test data.

Sample	Tensile stress-break (MPa)	Tensile strain-break	Maximum load (N)	Tensile strength (MPa)
1	9.70	0.04	0.93	11.63
2	4.99	0.03	0.51	5.10
3	10.84	0.03	1.02	11.33
4	8.66	0.17	0.74	10.57
5	20.97	0.02	2.57	21.42
6	9.37	0.04	1.01	11.22
7	10.74	0.16	1.25	12.50
Mean	10.75	0.07	1.15	11.97
Standard deviation	4.92	0.07	0.67	4.83

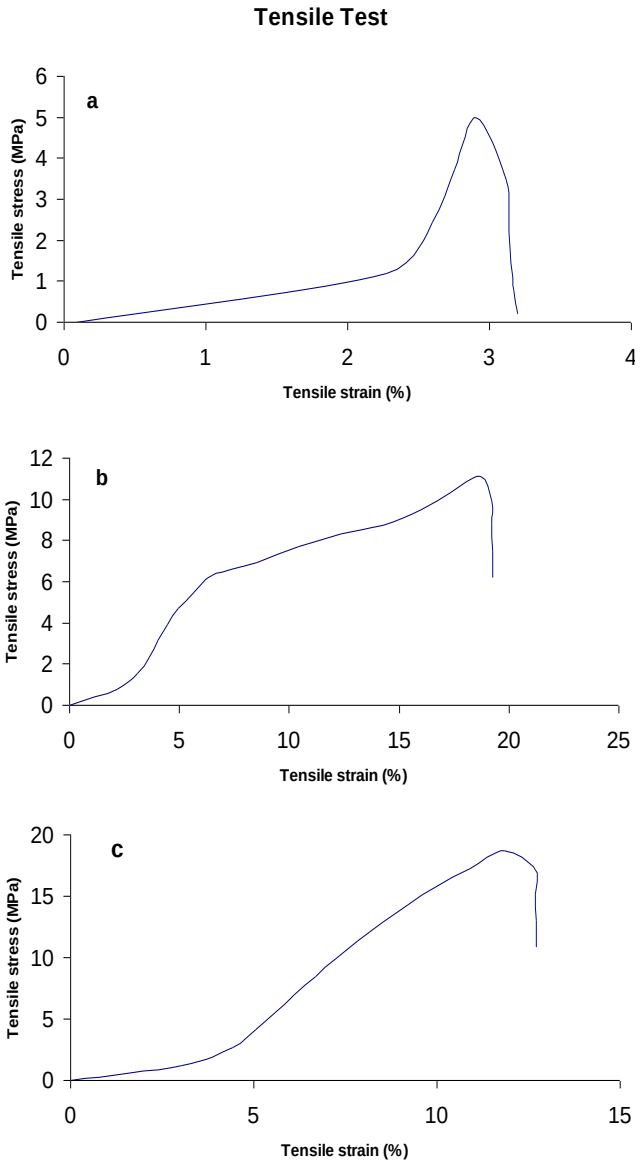

Figure 3. Examples of the stress-strain curve for (a) group 1 (welded collagen); (b) group 2 (welded collagen); and (c) group 3 (non-welded collagen).

Table 1 shows the selected data collected from the tensile test of the group 1 collagen samples. The tensile stress at the point of failure varied considerably for every collagen sample with the mean value of 10.75 (±4.92) MPa. The maximum load that could be sustained by the samples also showed variation with a mean of 1.15 (±0.67) N. The tensile strain at the point of failure had a mean of 0.07 (±0.07) MPa.

As previously mentioned, the behavior of the failure was also noted during the test for each of the collagen sample. For group 1, all of the samples, except for sample 4, failed in the middle of the welded part (depicted by the shaded area in Figure 2). Figure 3a shows an example of stress-strain curve from one of the group 1 samples (sample 2). Table 2 shows the selected data collected from the tensile test of group 2 collagen samples. The tensile stress and strain at the point of failure for every collagen sample had a mean value of 7.39 (±3.7) and 0.25 (±0.11) MPa, respectively. The maximum load that could be sustained by the samples showed a considerable variation, with a mean of 1.21 (±0.72) N.

Similar to the group 1 samples, all the samples in group 2, except for one (sample 8), failed at/or near the middle of the welded area. Sample 8, on the other hand, failed at the edge of the sample, around the area where the grip of the tensiometer was attached. Figure 3b shows an example of the stress-strain curve of a sample from group 2 (sample 11). Although, this is not a representative curve shape for all group 2 samples, a quick observation of the curve for all of the group 2 samples shows that there was a considerable difference in the general shape of the curves when compared with those of group 1 samples. Both of them may represent the welded collagen samples, but there appears to be a difference in the overall shape of the stress-strain curve. One possible explanation for this could be attributed to the difference in the timing of the welding and the testing processes for both groups. Group 1 samples were welded and tested on the same day, while there was a two-day gap between the welding and the testing processes

Table 2. Group 2 (welded collagen) tensile test data.

Sample	Tensile stress-break (MPa)	Tensile strain-break	Maximum load (N)	Tensile strength (MPa)
8	12.8	0.25	2.55	14.17
9	7.11	0.35	1.25	8.33
10	2.42	0.11	0.56	3.11
11	9.93	0.21	1.77	11.06
12	3.02	0.18	0.60	3.00
13	9.06	0.18	0.95	10.56
14	7.38	0.44	0.78	8.67
Mean	7.39	0.25	1.21	8.41
Standard deviation	3.7	0.11	0.72	4.13

Table 3. Group 3 (non-welded collagen) tensile test data.

Sample	Tensile stress-break (MPa)	Tensile strain-break	Maximum load (N)	Tensile strength (MPa)
1	17.13	0.14	1.61	17.89
2	18.81	0.20	2.33	21.18
3	4.45	0.38	0.85	5.31
4	8.27	0.15	1.10	9.17
5	12.98	0.25	1.83	14.08
6	19.44	0.32	3.01	27.36
7	16.26	0.28	2.10	17.50
Mean	13.91	0.25	1.52	16.07
Standard deviation	5.67	0.09	0.74	7.38

used for the group 2 samples.

Group 3 collagen samples represent the non-welded collagen, which will be used as a control in the analysis. The differences in the selected tensile test properties between the welded and the non-welded group of collagen samples were determined using statistical analysis. Table 3 shows the selected data collected from the tensile test of group 3 collagen samples. The tensile stress and strain at the point of failure for every collagen sample had a mean value of 13.91 (±5.67) and 0.25 (±0.09) MPa, respectively. The maximum load that could be sustained by the samples shows some variation with a mean of 1.52 (±0.74) N. Figure 3c shows an example of the stress-strain curve of a sample from group 3 (sample 1). Note that the general shape was similar when compared with the shape of the curve from the group 2 samples.

DISCUSSION

It was found that the statistical F-values for the stress and strain at failure point, the maximum load and the tensile strength sustained for all the three groups were 3.36, 9.14, 2.10 and 3.43, respectively. It can be seen, therefore, that only the tensile strain at the point of failure shows a significant difference ($P < 0.01$) between the sample groups. The tensile stress at the failure point, the maximum load and the tensile strength displayed no significant differences, even at $P < 0.05$. In order to determine which of the collagen groups had a significantly better strain value at point of failure, the range of values for each of the group needed to be determined. From Tables 1, 2 and 3, the following strain data can be observed:

1. Group 1: 0.02 to 0.17 MPa
2. Group 2: 0.11 to 0.44 MPa
3. Group 3: 0.14 to 0.38 MPa

It appears that collagen samples from groups 2 and 3 significantly differ from the group 1 samples in terms of the tensile strain value at the point of failure. It should be noted that there was a difference in the timing applied to the performance of the welding and the testing processes between groups 1 and 2, and this could well be the reason behind the differences seen above. Unless an exact experimental condition can be set, then a conclusion

with regards to the advantage or otherwise in welding the collagen cannot be made.

From these findings alone, it is very difficult to determine whether collagen is a possible candidate for an alternative sealant material. This is especially so, since there appears to be no difference in terms of the tensile strength of the samples, which is one of the main factors used to determine the usefulness of any material as a surgical sealant. Although, the strain level at failure point may be significantly different between the sample groups, inconsistencies with regards to the experimental conditions (during preparation of samples in groups 2 and 3, for example, as opposed to group 1) may well play a role in producing these results.

The findings from this study cannot be used as a sole reference in discussions involving the use of collagen as a sealant material. Nevertheless, the methodology for the experiments utilized within the study beginning from the preparation stage until the testing stage, could act as a sufficient basis for similar studies in the future.

Conclusion

Collagen may prove to be a possible alternative sealant biomaterial. However, further investigations need to be made in order to conclusively support or oppose the findings made in this study. Apart from investigating the mechanical properties of collagen, researchers must also look at other possible problems associated with the use of collagen, especially with regards to thermal damage of the tissue.

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