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An investigation of some factors influencing the in vitro assessment of mucoadhesion

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Abstract

The ultimate aim of this research is to develop in vitro systems that allow the prediction of in vivo performance of a mucoadhesive drug delivery system. In this novel approach a modified Dia-Stron rheometer was used that was capable of measuring the maximum force required, as well as the total work necessary, to detach a mucoadhesive containing disc from a model mucosal surface. Some of the factors that may affect the in vitro assessment of mucoadhesion were investigated, namely the method of measuring the adhesive strength, the nature of the mucosal surface, and the means of applying stress to the adhesive joint. A mucus gel, rat small intestine and, as a control, the non-adhesive surface of poly(vinyl chloride) tape were used as model mucosal surfaces. Test discs of various mucosa-adhesive materials were left in contact with the model mucosal surface for 2 min in a pH 6.0 isotonic phosphate buffer at 37°C, prior to testing. The model mucosal surface was then pulled away from the test disc at a rate of 2 mm min^{-1} until adhesive failure occurred. The attempt to apply and measure shear forces met with limited success. The results obtained on application of tensile stresses indicated that both the maximum detachment force and the total work of adhesion provided very similar measures of the relative adhesive strength for each test material. The discs were found to adhere to the control poly(vinyl chloride) tape stronger than rat's small intestine, with the weakest adhesion being obtained with the mucus gel. It was concluded that these mucoadhesive materials on hydrating are capable of adhering to a variety of different surfaces and a specific mucus/mucoadhesion interaction is not an important factor.

Keywords: Mucoadhesion; Mucosa adhesion; Mucoadhesive; Bioadhesion; Tensile stress; Shear stress; Work of adhesion; Detachment force

1. Introduction

The use of bioadhesive polymers and copolymers as means of delivering therapeutically active drugs, including proteins and peptides, to or via mucous membranes has been the focus of attention in recent years (Gu et al., 1988; Junginger, 1990; Jiménez-Castellanos et al., 1993a; Moës,

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1993). The term 'bioadhesion' is used to define the attachment of synthetic or natural macromolecules to a biological substrate. If this substrate is a mucous membrane, covered with a coating of mucus, bioadhesive interactions are said to occur primarily with the mucus layer and this phenomenon is referred to as 'mucoadhesion' (Gu et al., 1988).

Mucosal-adhesive materials have been investigated and identified in previous work (e.g., Chen and Cyr, 1970; Smart et al., 1984). These are generally hydrophilic macromolecules that contain numerous hydrogen bond forming groups, notably carboxyl, hydroxyl, amide and amine groups, and will hydrate and swell when placed in contact with an aqueous solution. These materials need to hydrate to become adhesive but overhydration usually results in the formation of a slippery mucilage and a loss of the adhesive properties.

The first step in the selection of a mucoadhesive material for controlled drug delivery is to determine whether the properties of the system are suitable for the intended application. Testing is essential for the development, characterisation, and proper use of the mucoadhesive delivery system. However, it is not easy to extrapolate the behaviour of a bioadhesive system from an in vitro test to its performance in vivo. This is because in vitro testing is generally made under a controlled environment, different to the constantly varying conditions observed in vivo. The use of a tensiometers to measure the strength of a mucoadhesive dosage form has been reported in numerous publications (e.g., Smart et al., 1984; Park and Robinson, 1985; Ponchel et al., 1987; Bottenberg et al., 1989; Gursoy et al., 1989; Lehr et al., 1989; Lejoyeux et al., 1989; Smart, 1991; Chen and Hwang, 1992; Dyvik and Graffner, 1992; Thermes et al., 1992). The information on the relative adhesive properties of various materials can show considerable discrepancies between studies, which is probably related to the differing experimental conditions. The general aim of this series of studies is to devise a standard tensiometer test system that allows the in vivo performance of a dosage form to be predicted from in vitro studies. In this work the following factors that may affect the adhesive forces in an in vitro system were considered:

The means of assessing the adhesive force. The 'work of adhesion' and the force required to produce joint failure (the maximum detachment force) have both been used to assess the strength of the adhesive joint, It has been proposed that the work of adhesion is the best method of quantifying mucoadhesion (Ponchel et al., 1987).

The nature of the mucosal surface. It has been proposed that mucoadhesion occurs by a process of wetting and then interpenetration of the mucoadhesive polymer with the mucus gel. (Duchene et al., 1988). If there is a specific interaction between the mucoadhesive and mucus gel then the presence of mucus would be predicted to be important in the formation of a stable adhesive joint. A mucus gel, and a model mucosal surface used in several previous studies (Smart, 1991, 1992), were used as test surfaces. As a control a non-porous material (PVC tape) was used to assess the general adhesive properties of these materials.

The means of applying force to the adhesive joint. Tensile testing is usually used to assess adhesive joint strength. However, the mucoadhesive dosage form is more likely to be subjected to shear stresses when placed in vivo, e.g., within the gastrointestinal tract. Therefore, in order to determine whether the direction of the force applied to the mucoadhesive joint could influence the mucoadhesive strength of the test system a novel test system was investigated.

2. Materials and methods

2.1. Materials

Carbopol 934P (C934), Carbopol 2984 (C2984) and Pemulen TR-1 (Pem. TR-1) were obtained as gifts from B.F. Goodrich, Hounslow, UK, analar sodium dihydrogen orthophosphate (dihydrate), disodium hydrogen orthophosphate (dihydrate), sodium chloride, sodium alginate, and tragacanth were purchased from BDH Chemicals Ltd, Poole, UK, sodium carboxymethyl cellulose (viscosity 3000–6000 cP, 1% aqueous solution) (NaCMC),

poly(ethylene oxide) (PEO) with a molecular mass of 4000 kDa, and hydroxypropyl cellulose (HPC) with an average molecular mass of 1000 kDa were obtained from Aldrich Chemical Co. Ltd, Gillingham, UK, carrageenan type II, and karaya gum were purchased from Sigma Chemical Co. Ltd, Poole, UK, hydroxypropylmethyl cellulose (Methocel K100 M) (HPMC) was obtained from Colorcon Ltd, Orpington, UK, and 13 mm diameter Whatman cellulose nitrate membrane filters with a pore size of 0.45 mm were purchased from Fison Scientific Equipment, Loughborough, UK.

2.2. Preparation of the buffer solution

Isotonic phosphate buffer at pH 6.0 was prepared by dissolving 9.03 g l^{-1} sodium dihydrogen orthophosphate dihydrate, 1.59 g l^{-1} disodium hydrogen orthophosphate dihydrate, and 5.17 g l^{-1} sodium chloride in purified water.

2.3. Preparation of the test discs

50 mg samples of the test materials were compressed into 6.2 mm diameter, flat-faced discs in a Specac infrared press, using a 1 Tonne force for 5 s.

2.4. The mucoadhesion apparatus

The Dia-Stron rheometer (Dia-Stron Ltd, Andover, UK) has been designed primarily for application in cosmetic and hair research. However, the instrument may be modified for other applications. The rheometer measures the force applied to the sample during uni-axial extension or compression. The degree of extension and the rate are pre-determined prior to initiating the test. Furthermore, the data collected are stored and available for plotting or transfer to a personal computer. The computer software provided further enhances the utility of the instrument by facilitating data handling and the calculation of stress-strain properties. The maximum breaking force, as well as the work expended in extending or compressing the sample are the main features which could be obtained from the software provided. The rheometer had to be modified for the tensile and shear studies conducted on the mucoadhesive discs. A jacketed water bath made of clear perspex was built 'in house'. This was fitted between a fixed sensor arm, measuring the extension force and distance applied on the sample, and a moving platform (Fig. 1).

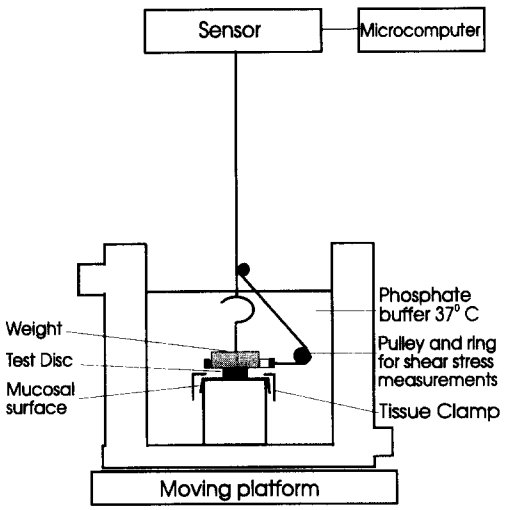

Fig. 1. Schematic diagram of the mucoadhesion apparatus showing tensile and shear arrangements.

2.5. Preparation of test surfaces

Mucus gels: 10 g batches of crude mucus were obtained by scraping three to four hog stomachs which had been separately freshly frozen at −20°C then thawed at room temperature (20°C) before use. These were gently blended to ensure homogeneity and then used without further treatment. The final preparation was evaluated to ensure that it had appropriate viscoelastic rheological properties. The percentage dry weight of 'solids' present in the batches of crude mucus gel produced was determined by leaving a small portion (0.5 g) of mucus in pre-weighed open glass vials at 50°C for 48 h and found to be between 8 and 9% in all cases.

100 mg samples of the mucus gels were individually weighed and evenly spread over 13 mm diameter Whatman membrane filters to give an average depth of 0.75mm. The mucus coated

filters were then allowed to stand for 2 min prior to testing.

The mucosal surface of rat's small intestine: The rat small intestine is relatively free of intestinal content, and provided a macroscopically flat and uniform surface. The middle section, discarding the first 40–50 mm at either ends of fresh intestine from male Wistar rats, was frozen until required to inhibit muscle contraction (Smart, 1991). This was cut into 3 cm lengths, opened longtitudinally to expose the inner mucosal surface, then gently washed with pH 6.0 isotonic phosphate buffer prior to testing. A preliminary histological study indicated that damage to this tissue from freezing and thawing was minimal, and a layer of mucus was present on the mucosal surface.

The control: The non-adhesive side of PVC tape was used as an inert, mucus free, surface.

2.6. Experimental

The mucus coated filters and the sections of the rat intestine were individually mounted on a platform (within the jacketed water bath) and secured in place, using a plastic cap, exposing an 11 mm diameter circle of the test surface. PVC tape was attached on the platform, using its adhesive side. All the surfaces tested were allowed to equilibrate in the pH 6.0 isotonic phosphate buffer for 1 min, at 37°C.

50 mg test discs were individually attached to a 1.5 g weight, using a cyanoacrylate adhesive. The tensile studies were conducted by suspending the 1.5 g weight with attached test disc, from the force and position sensor arm of the Dia-Stron rheometer. This was lowered onto the adhesive surface and left for 2 min. The moving platform was then lowered at a rate of 2 mm min^{-1} and the maximum detachment force (MDF) and the total work of adhesion (TWA, the area under the force/elongation curve) calculated, using the software provided.

The system to evaluate mucoadhesion on application of shear stresses was similar to that described by Leung and Robinson (1988). When conducting the shear stress studies the round plastic cap, securing the adhesive surface, had to be elongated on one side, providing sufficient distance for the test disc to be detached during shear studies. The test discs were individually attached to a 1.5 g weight, using the cyanoacrylate adhesive, lowered into the pH 6.0 buffer (at 37°C) and placed in contact with the adhesive surface for 2 min. A brass ring with an internal diameter of 10.5 mm was then placed over the 1.5 g weight. The ring was connected to the force and position sensor of the rheometer via a pulley system (Fig. 1). After 2 min contact (between the test disc and the adhesive surface) the platform was lowered at a rate of 2 mm min^{-1} and MDF and TWA calculated.

As a control, the tensile and shear stress experiments conducted on the various adhesion surfaces used were completed without the test discs.

3. Results

3.1. Tensile studies

A typical force elongation curve is shown in Fig. 2. The peak value represents the maximum detachment force and the area under the curve is the total work of adhesion.

It is evident that the adhesive strengths (both the MDFs and the TWAs) are greatest for the PVC coated platform (over twice that of the rat intestinal surface in most cases), and least for the

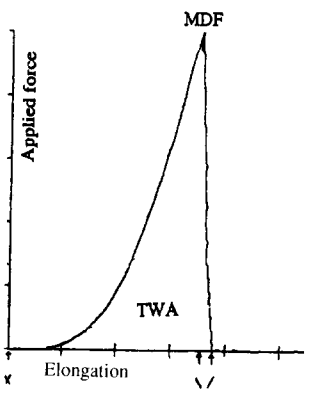

Fig. 2. A typical force elongation curve output from the Diastron rheometer on application of tensile stress.

Table 1
Rank order of adhesion to mucus for various mucoadhesive materials subjected to tensile stresses in a pH 6.0 isotonic phosphate buffer ($n = 5$)

Test material	Maximum detachment force (mN) (S.D.)	Total work of adhesion (μJ) (S.D.)
Na alginate	250.07 (54.80)	186.40 (49.52)
Carrageenan II	198.30 (29.66)	121.28 (16.96)
NaCMC	179.26 (49.30)	142.34 (54.00)
PEO	119.25 (21.30)	72.44 (12.21)
Karaya gum	115.30 (39.60)	71.28 (25.81)
C2984	96.30 (13.13)	61.66 (10.63)
Pemulen TR-1	90.22 (40.10)	66.30 (28.83)
C934	89.83 (32.20)	67.46 (35.41)
Tragacanth	64.70 (6.83)	45.18 (7.91)
HPMC	49.23 (6.22)	36.20 (4.58)
HPC	29.42 (7.04)	25.46 (5.76)
Control (no disc)	16.28 (2.74)	9.88 (3.26)

Table 2
Rank order of adhesion to rat intestinal mucosa obtained with various mucosa-adhesive materials subjected to tensile stresses in a pH 6.0 isotonic phosphate buffer ($n = 5$)

Test material	Maximum detachment force (mN) (S.D.)	Total work of adhesion (μJ) (S.D.)
PEO	917.31 (297.51)	532.60 (249.40)
NaCMC	699.21 (115.20)	359.60 (77.00)
C2984	649.20 (225.90)	239.14 (86.34)
C934	623.70 (119.80)	228.20 (50.20)
Carrageenan II	527.60 (241.15)	216.20 (119.50)
Pemulen TR-1	313.80 (129.40)	123.16 (40.27)
Na alginate	196.13 (47.16)	71.32 (14.15)
Tragacanth	182.21 (35.62)	41.92 (13.67)
Karaya gum	169.65 (16.31)	60.02 (7.60)
HPMC	105.91 (40.83)	35.70 (17.49)
HPC	35.31 (5.76)	7.39 (1.80)
Control (no disc)	13.73 (2.08)	4.70 (3.40)

mucus gel (less than half that of the rat intestinal surface in most cases) (Tables 1–3). A reasonable linear relationship was obtained when comparing the mean MDF and TWA for each test material using the rat intestinal surfaces (Fig. 3). Although some of the data showed a high degree of variation, the Pearsons correlation coefficient (r) was calculated as an indicator of linearity and found to be 0.97. A similar degree of linearity between the mean TWA and MDF was observed when mucus and PVC were used as the test surfaces ($r = 0.98$ and 0.96, respectively).

The rank order of adhesiveness for the rat small intestine relative to the PVC coated film was very similar (Fig. 4 and 5), giving r values of 0.93 for the MDF and 0.96 for TWA. However, there was little correlation of the rank orders between mucus and rat intestine (Fig 6) and also mucus and PVC (e.g., $r = 0.44$ and 0.33, respectively, for the MDFs). A material like sodium alginate for example, which has a high adhesive

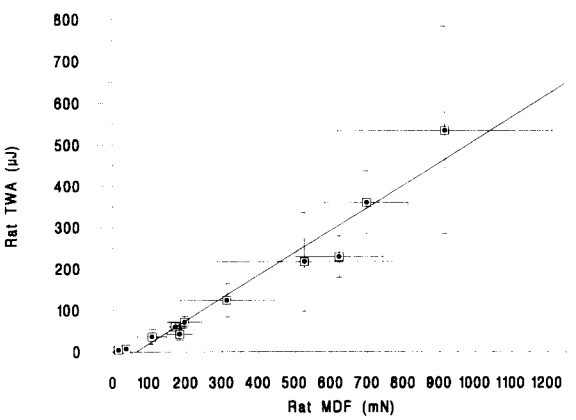

Fig. 3. Correlation between the maximum detachment force and total work of adhesion for the rat small intestinal surface ($n = 5$, S.D. bars).

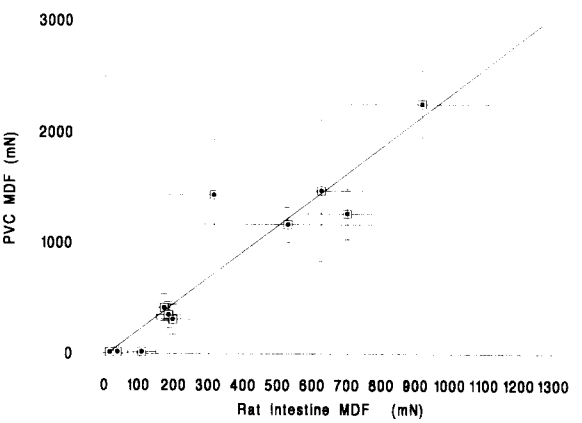

Fig. 4. Correlation between the maximum detachment forces obtained for various putative mucoadhesive materials using PVC and rat small intestine as model surfaces ($n = 5$, S.D. bars).

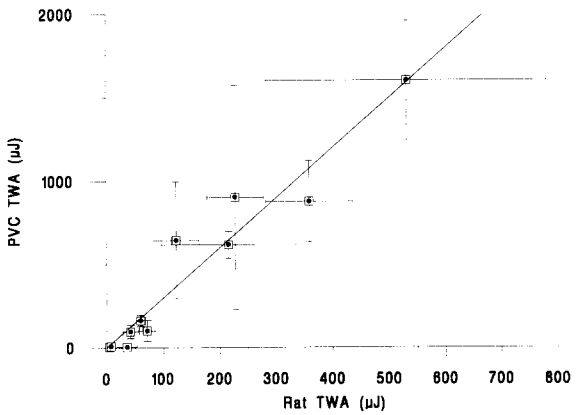

Fig. 5. Correlation between the total work of adhesion for various putative mucoadhesive materials using PVC and rat small intestine as model surfaces ($n = 5$, S.D. bars).

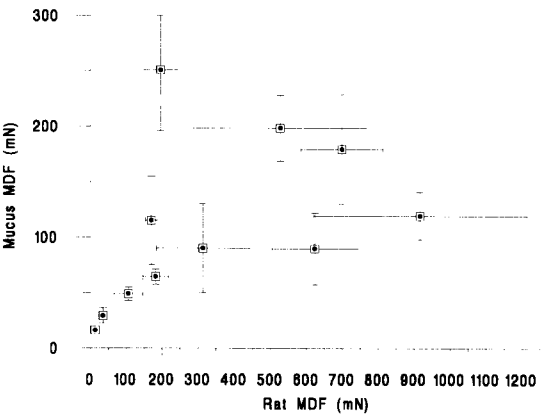

Fig. 6. Correlation between the maximum detachment forces obtained for various putative mucoadhesive materials using mucus and rat intestine as model surfaces ($n = 5$, S.D. bars).

ranking with mucus, had a lower adhesive ranking with both PVC tape and the rat intestine.

3.2. Shear testing

This system of shear testing was found to provide several problems, notably:

(1) Frictional forces within the pulley system were found to make a significant contribution to the detected adhesion force. These effects were found to be variable and so could not be removed from the adhesive force values by calculation.

(2) A clear adhesive joint failure was often not evident as the joint would appear to break then reform. Therefore, calculation of the work of adhesion was not always possible.

However, differences in the forces detected using these two systems was apparent and this will need to be investigated further.

Table 3
Rank order of adhesion to PVC tape obtained with various materials subjected to tensile stresses in a pH 6.0 isotonic phosphate buffer ($n = 5$)

Test material	Maximum detachment force (mN) (S.D.)	Total work of adhesion (μJ) (S.D.)
PEO	2255.50 (301.10)	1602.00 (359.40)
C934	1474.90 (642.40)	901.00 (672.55)
Pemulen TR-1	1435.70 (502.40)	644.40 (350.37)
NaCMC	1265.10 (231.80)	877.40 (243.65)
C2984	1257.20 (390.50)	553.80 (249.37)
Carrageenan II	1167.00 (160.40)	617.00 (80.18)
Karaya gum	411.90 (121.70)	161.20 (33.54)
Tragacanth	347.15 (119.02)	95.40 (39.74)
Na alginate	305.97 (135.40)	101.42 (63.45)
HPMC	14.91 (5.86)	3.91 (0.79)
HPC	13.14 (5.62)	7.11 (4.08)
Control (no disc)	10.98 (1.07)	4.23 (1.90)

4. Discussion

It is evident that the work of adhesion and maximum detachment force appears to be providing the same information on bond strength in this study, with the relative rank orders of adhesiveness being similar. The maximum detachment force measures the maximum force an adhesive joint can withstand before breaking (Fig. 2). This will depend on the strength of the weakest component of the joint, which in this case could be the mucus gel or the hydrating dosage form. This situation is further complicated by the fact that mucus and the gelling polymers can both show viscoelastic rheological properties. The rate of application of the force may therefore affect the maximum detachment force obtained, depending on whether these materials show elastic or vis-

cous liquid deformation under these conditions. The total work of adhesion is the area beneath the force elongation curve (Fig. 2). Again this measure of adhesion will depend on the elasticity of the mucus and gelling dosage form, the more 'elastic-like' these are the greater the elongation. Because of the complexity of these systems it is unlikely that one measure of adhesive strength would be better than any other as an indicator of adhesive performance, and perhaps a re-evaluation of the force elongation curves would provide more useful information. Joint failure was observed to be an adhesive failure at the interface only with the weakest adhesives, and was normally a cohesive failure in one of the adhering surfaces. Therefore, it is proposed that the term 'total work of detachment' would be more appropriate to describe this measure of adhesive joint strength.

It is clear that these materials become adhesive on hydrating and adhered most strongly to the inert control surface. In previous work we have demonstrated that mucoadhesive materials can dehydrate mucus gels (Mortazavi and Smart, 1993) and interact with mucus glycoproteins to produce gel strengthening by a proposed method of intermacromolecular complex formation (Mortazavi and Smart, 1994). Other workers have investigated the role of surface properties (i.e., the surface energies) in mucoadhesion (Lehr et al., 1993; Esposito et al., 1994). It was suggested in each case that these factors would be important in the formation of a strong mucoadhesive joint. In this study the presence of mucus or mucous glycoproteins would appear not to be necessary for adhesion. In fact the more mucus present the weaker is the adhesive force, which is consistent with the lubricant role of mucus within the gastrointestinal tract. With the mucus gel surface, joint failure resulted from a cohesive failure within the mucus, deposits of which were visible on both the test disc and membrane filter. However it must be noted that the physicochemical properties of the mucus samples used in this study may differ from that present on a target mucosal surface. The rat intestinal surface gave a rank order correlation for adhesive strength closest to the control PVC tape where factors like hydrogel interpenetration (Jabbari et al., 1993), intermacromolecular complex formation and dehydration clearly could not occur. We have proposed that the process (the stages in the development) of mucoadhesion may differ under varying conditions, e.g., with fully or partially hydrated mucoadhesives, with mucosae with substantial or limited mucus layers, whether the dosage form can be directly places onto the target mucosa or if a preliminary physical adsorption is required (Mortazavi and Smart, 1993), and this would be consistent with the differences seen in this study. In future a consideration of the general adhesive properties of a test material against standard, well characterised, inert surfaces may be a good starting point for the investigation of mucoadhesion.

The shear stress measurements were found to be surprisingly difficult to complete with the apparatus as designed. The pulley system introduced a variable amount of error into the system which, despite several design modifications, proved difficult to eliminate or control. Pulling the adhesive joint horizontally also meant the gravitational forces encouraged the test disc to readhere to the surface, and frictional effects could not be separated from adhesive effects. The difficulty in measuring shear stresses encountered in this study may explain the comparatively limited work published using this technique (e.g., Chen and Cyr, 1970; Leung and Robinson, 1988; Jiménez-Castellanos et al., 1993b) despite this being the type of stress most likely to be encountered by a dosage form in vivo. However, there was some evidence of substantial differences between the adhesive forces detected using tensile and shear stresses and in order to fully investigate this, fundamental design modifications to eliminate both the pulley system and re-adhesion due to gravity are now under investigation.

The Dia-Stron rheometer provided a good method for the investigation of mucoadhesion, allowing the calculation of both the maximum detachment force and work of adhesion. One limitation of this apparatus is the relative inflexibility of the software given the rather unusual use it has been put to in this particular study. Future work will concentrate on developing a more flexi-

ble system that will allow the assessment of both tensile and the more relevant shear stresses.

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