An advanced 3D monofilament biosuture

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Introduction

Sutures are one of the most widely used medical devices with employment in over 12 million procedures per year globally.[1] Yet, the ideal suture material does not exist. Over the years scientists and surgeons alike have set out to find a suture material that is biocompatible, easy to handle, does not cause unnecessary tissue damage and creates an optimal environment for wound healing.[2] This has led to the discovery of numerous suture materials ranging from silk and catgut in the early 1800s to synthetic polymers such as polylactic acid and polyglycolide that are currently in use.[3] Sutures on the market today are associated with distinct disadvantages that threaten the success of the procedures they are used in. For example, sutures consisting of polyglactin have been found to cause severe tissue damage and necrosis through the release of acidic degradation products that result in strong inflammatory responses.[4] Additionally, most suture materials are multifilament or braided materials that tend to cut through tissues causing cellular damage and extending the time of wound healing.[5] Suture-related complications have been implicated in numerous clinical problems such as hematoma formation, pulmonary embolisms and false aneurysms. A retrospective study done by Starr *et al.*, found that 26 of 39 false aneurysm cases could be directly ascribed to insufficiencies in the suture material.[6] There is, therefore, a clear yet understated need for a superior suture material that can limit suture-related complications and improve the success rate of surgical procedures.

The current project set out to develop a novel 3D monofilament biosuture, consisting of natural polymers only. The new suture material is fully biocompatible, biodegradable, nonimmunogenic and does not release acidic degradation products into the environment. Unique 3D printing technology and computer-aided design (CAD) was used to obtain the monofilament suture platform with a smooth, uniform surface that allows the biosuture to easily glide through delicate tissues without causing unnecessary harm.

Materials and methods

Materials and biosuture fabrication

Sodium alginate and pectin from citrus peel was purchased from Sigma-Aldrich (St. Louis, MO, USA). The mannuronic acid and guluronic acid concentration ratio (A_{1026}/A_{1080}) of sodium alginate was calculated to be 1.52 as deducted from Fourier Transform Infrared Spectrometry (FTIR) data and described in literature.[7] Barium chloride ($BaCl_2$) was employed as crosslinking agent and glycerol as plasticiser (LabChem, Foundershill, Johannesburg, SA).

A novel bioink was created from a homogenous hydrogel consisting of two natural polymers namely sodium alginate and pectin from citrus peel. The two polymers were dissolved separately through mechanical stirring at 35°C for one hour. The two solutions were then combined, glycerol added, and the resulting solution vigorously stirred until a homogenous hydrogel was obtained. The hydrogel was immediately transferred to a clear, sterile 3D printing syringe that was loaded onto the 3D Bioplotter® (EnvisionTEC, GmBH, Germany). The hydrogel was now used as a novel bioink by printing it, according to the intercalated architectural design, into a $BaCl_2$ solution, that allowed for *in situ* crosslinking and suture formation. The printed biosutures were left to crosslink for 24 hours after which they were washed five times with deionised water and airdried in a fume hood for six hours.

Physiochemical analysis of the biosuture material

The FTIR spectra of the biosuture material was compared to that of the pristine polymers and analysed for specific chemical transitions that would confirm crosslinking of the polymers with $BaCl_2$. FTIR parameters were set as follows: 64 scans and a wavenumber range of 4000-650 cm^{-1} using a PerkinElmer Spectrum 2000 ATR-FTIR spectrometer (PerkinElmer 100, Llantrisant, Wales, UK).

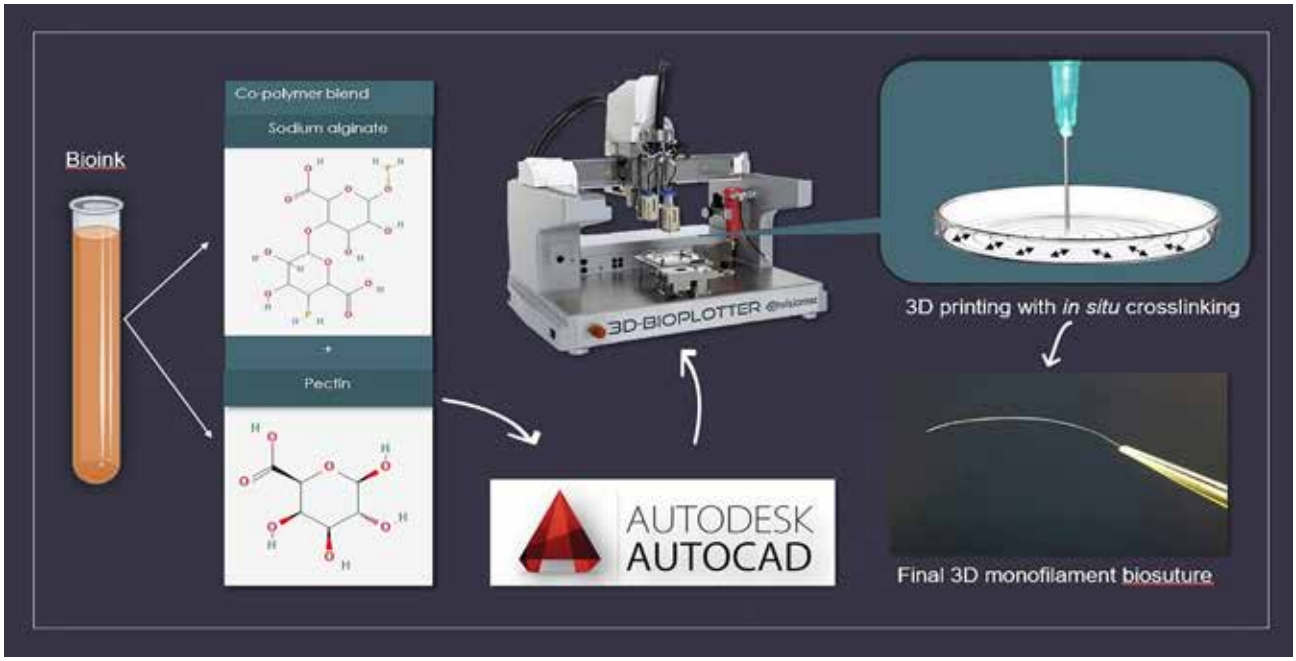

Figure 1: Schematic representation of the biosuture fabrication process

Thermal analysis of the biosuture material

The thermophysical properties of the fabricated biosuture and the pristine polymers were determined using a differential scanning calorimeter (DSC) (Mettler Toledo, DSC, STARe System, Swchwerzenback, ZH, Switzerland). Samples were heated over a temperature range of 20-400°C at a heating rate of 10°C/min under a constant N_2 gas atmosphere. DSC curves were plotted as heat flow against temperature and studied to determine the influence of crosslinking on the thermal properties of the materials.

Tensile strength testing of the biosuture material

Uniaxial strain tests were conducted on biosuture samples using the BioTester 5000 (CellScale, Waterloo, ON, Canada). Tests were done according to methods described in the United States Pharmacopoeia (USP). Briefly, the BioTester 5000 was balanced, calibrated, and the clamps moved 6.5 cm apart. Biosuture samples, measured at 7 cm in length and 100 µm in diameter, were secured between the two clamps of the BioTester 5000. Once mounted, the biosuture samples were stretched at a constant rate of 14cm/min until breakpoint as specified by the USP. Stress-strain graphs were obtained, and the tensile strength calculated using Equation 1:

$$\text{Tensile strength} = \frac{F_{max}}{t \times w}$$

Where F_{max} is the load failure (force at which the suture breaks), t is the initial biosuture thickness, and w is the initial width of the biosuture.

Evaluation of the surface morphology of the biosuture material

To study the surface properties of the biosuture material, images were taken with the Scanning Electron Microscope (SEM, FEI Nova Nanolab 600 FEG-SEM/FI, Hillsboro, USA) at 1000x magnification. Before analysis, the biosuture samples were mounted onto aluminium stubs using carbon adhesive tape and coated with two coats carbon and one coat palladium/gold at an 80:20 ratio.

In vitro degradation analysis of the biosuture material

Five samples of the biosuture material were weighed (1.5 mg each) and transferred to vials containing 5 ml PBS (pH 7.4). The vials were placed into the orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd. Hahistadrut, Holon, Israel) set at a speed of 50 rpm and maintained at 37°C. The degree of degradation was evaluated every 24 hours by weighing each sample on a micro analytical balance (MS-TS Analytical balance, Mettler-Toledo S.A. Zaventem 1932, Belgium) over a period of 60 days. The *in vitro* degradation of the biosuture was calculated using Equation 2:

$$\text{Degradation (\%)} = \frac{(M_0 - M_d)}{M_0}$$

Where M_0 is the starting weight of the biosuture sample before degradation and M_d is the dry weight of the biosuture sample on the measurement day.

Results and discussion

Biosuture fabrication

3D monofilament biosutures, consisting of two natural polymers namely sodium alginate and pectin from citrus peel, were successfully obtained through extrusion, using the 3D Bioplotter® (EnvisionTEC, GmBH, Germany).

Physiochemical analysis of the biosuture material

The FTIR spectra of pristine sodium alginate revealed two characteristic bands at 1590 cm^{-1} and 1402 cm^{-1} respectively

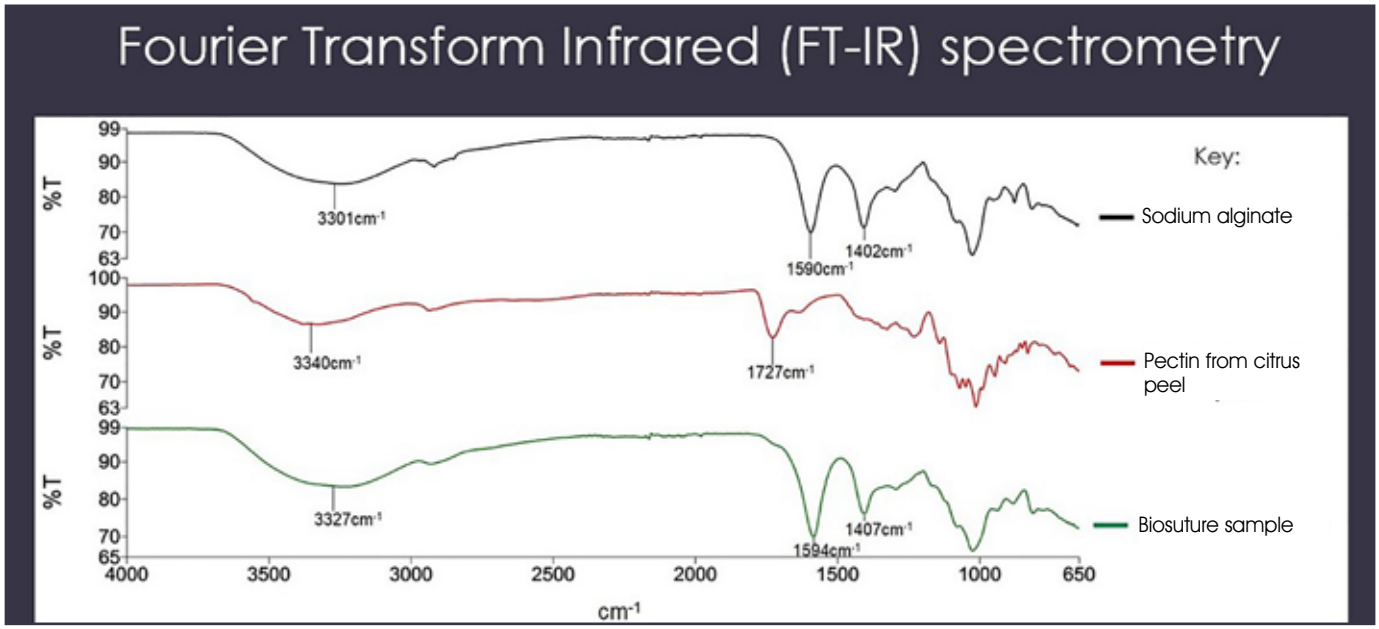

Figure 2: FTIR curves of sodium alginate, pectin from citrus peel and biosuture sample taken with a PerkinElmer Spectrum 100, FT-IR Spectrometer

(Figure 2). These bands can be attributed to the symmetric and asymmetric stretching vibrations of the carboxylate salt of sodium alginate that is often used to describe this polymer and its derivatives. In the biosuture sample, a slight downshift was observed in the wavenumber of these bands to 1 594 cm^{-1} and 1 407 cm^{-1} respectively. This downshift confirms the effective crosslinking of sodium alginate while the slight alteration in wavenumber points to a mere electrostatic interaction between Ba^{2+} and the carbonyl group of sodium alginate.[8]

The FTIR spectrum of pristine pectin also suggests crosslinking of this polymer as the absorption band observed at 1 727 cm^{-1} has shifted in the biosuture sample spectrum. This band, which can be attributed to the ester bond in the molecule backbone of pectin, is not visible in the biosuture spectrum, possibly due to a downshift in the wavenumber after crosslinking, that now causes the band to overlap with the stretching vibrations of the carboxylate groups of sodium alginate.[10]

Thermal degradation analysis of the biosuture material

Differential scanning calorimetry (DSC) is a useful technology to study the thermal behaviour of materials, which is correlated to their structure and hydrophilic properties. The DSC curve of sodium alginate shows an endothermic peak around 100°C that can be attributed to the release of loosely bound water from the polymer surface. In the biosuture sample curve the endothermic dehydration band is flattened after cross-linking which points to the strong interaction of barium-carboxylate pairs with the hydrating water molecules. A sharp endothermic band is also witnessed at 218°C that may correspond with cleavage enthalpies of the barium-carboxylate bonds within the biosuture material. Such a sharp endothermic band suggests that the cross-linked material has a highly ordered molecular structure which is further verified by the higher decomposition temperature of 230°C compared to the 210°C of the pristine polymers.

Tensile strength of the biosuture material

Mechanical strain analysis was performed using the BioTester 5000 (CellScale, Waterloo, ON, Canada), which is equipped with image analysis software. The USP specifies two methods for suture tensile testing namely, the straight tensile test and the knot tensile test. Both these tests were performed using the BioTester 5000 and the resultant tensile test calculated to be 19.8N for the straight tensile test and 2.2N for the knot tensile test. According to the USP an absorbable suture, in the size range of the fabricated biosuture, should have a minimum knot strength of 1.76N. The tensile strength of the biosuture material therefore exceeds the minimum value specified by the USP, suggesting that the biosuture material will have sufficient tensile strength to support

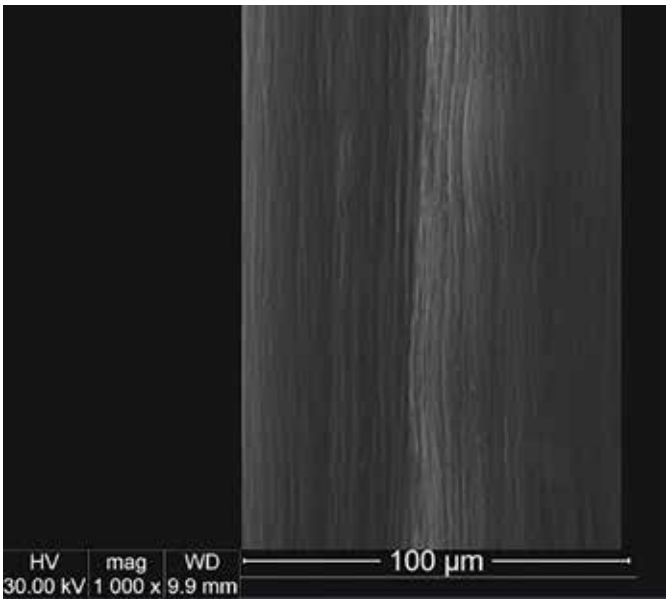

Figure 3: Image of the biosuture taken with the Phenom Microscope (FEI Nova Nanolab 600 FEG-SEM/FI, Hillboro, USA)

a healing wound.

Surface morphology of the biosuture material

SEM images revealed a smooth, uniform suture surface with a fibrous nature (Figure 3). Such a suture morphology will experience little resistance when passing through tissues and will not cause the cellular damage usually experienced with multifilament sutures. Images were also used to determine the precise suture diameter which came to an average of 100 µm and corresponds with a 6-0 suture size.

In vitro degradation analysis of the biosuture material

An *in vitro* degradation study was done to determine how the biosuture material will degrade under physiological conditions. The first sign of weight loss was detected on day 28, after which gradual degradation took place up to day 60 where the biosuture material reached around 35% of the initial weight. This suggests that the biosuture material will provide constant and reliable support to a wound for up 28 days, which is sufficient time for most wounds to heal. The study also indicates that the biosuture material is indeed biodegradable and will not remain in the body indefinitely, thereby preventing any unwanted irritation or chronic inflammation from occurring.

Conclusion and future prospects

A novel 3D monofilament biosuture has been fabricated from natural polymers only. The biosuture has an optimal degradation profile as deducted from the *in vitro* degradation study, superior tensile strength as indicated by the knot tensile test and optimal surface morphology as revealed by SEM images. FTIR analysis and DSC thermal degradation curves illustrate the effective crosslinking and increased stability of the biosuture material.

This new suture material with its ease of fabrication, high adaptability and inherent biocompatibility can help overcome suture-related complications and improve the success rate of numerous surgical procedures. Furthermore, this new biosuture material holds the exciting potential of drug-loading that can be used to achieve both burst or sustained drug release and help overcome additional problems, such as infection, that are often experienced in surgery today.

Acknowledgements

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