Color changing bioadhesive barrier for peripherally inserted central catheters

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Key words: *Intravenous catheter, Polycaprolactone bioadhesive, Antibacterial barrier, Infection colorimetric indicator, UVA crosslinking.*

SUPPLEMENTARY INFORMATION

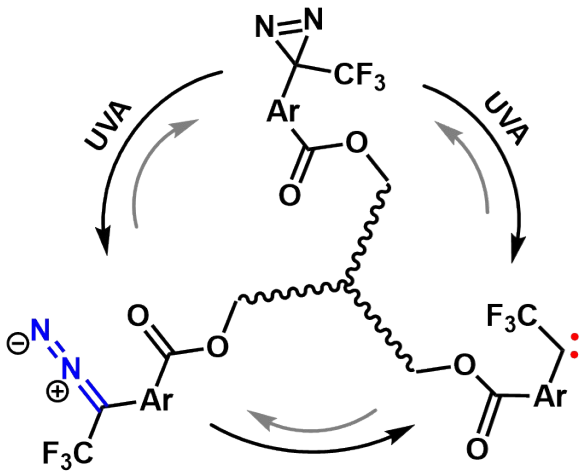

Figure S1. Schematic presentation of diazirine-grafted polycaprolactone tetrol (CG^-): The formulation undergoes liquid-to-biorubber transition by carbene insertion (both internal crosslinking and covalent insertion onto skin surface) activated by UVA (365 nm) light; diazoalkane by-product is present after diazirine photoreaction upon UVA activation.

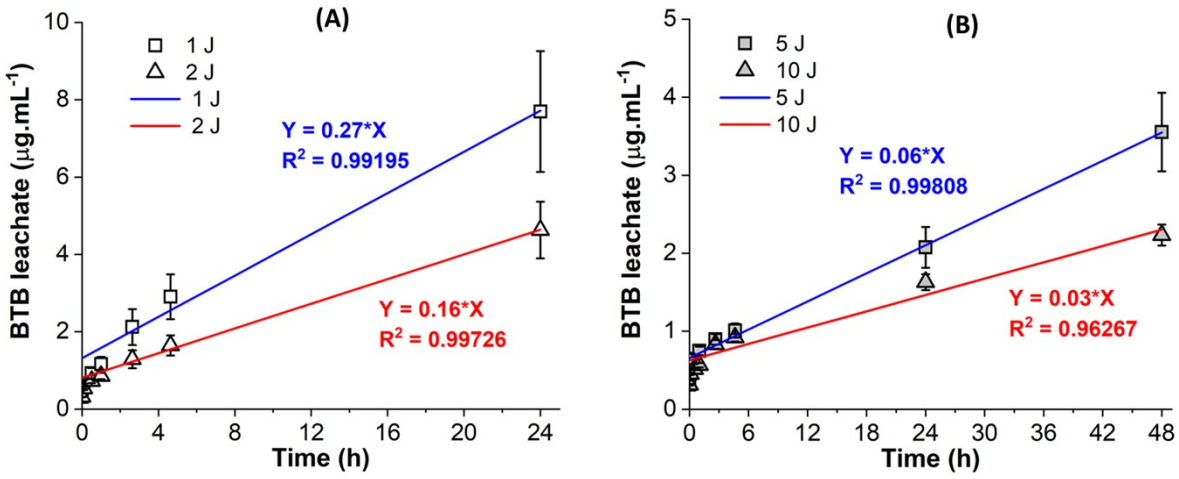

Figure S2. Linear bromothymol blue (BTB) release kinetics from UVA crosslinked CathoGlu (CG^+; BTB concentration = 1%; w/w) in PBS (pH = 8) - BTB concentrations vs time, released from CG^+ crosslinked at the total UVA doses of: (**A**) 1 J and 2 J; (**B**) 5 J and 10 J (n = 3).

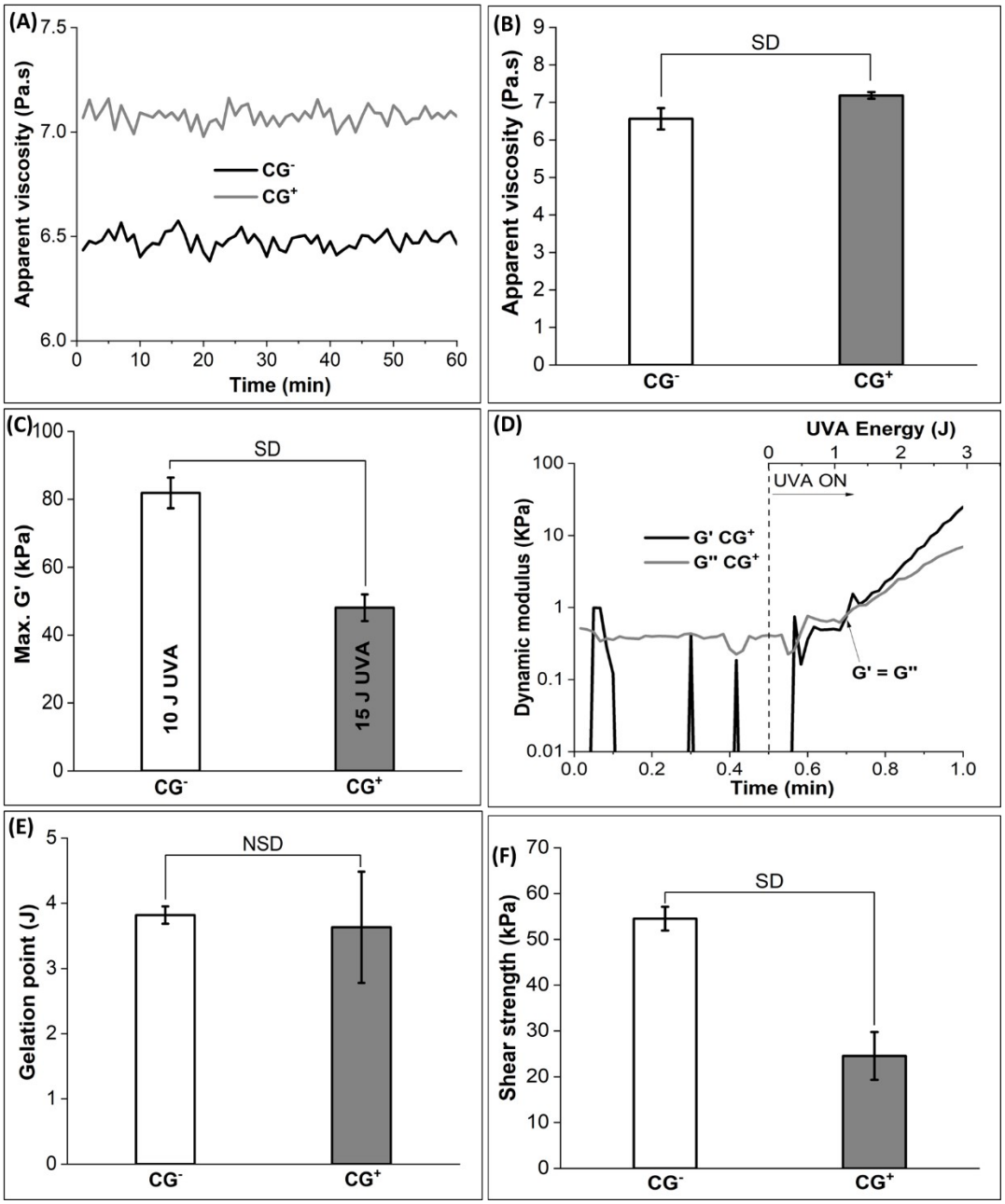

Figure S3. Rheological properties of CathoGlu measured with parallel probe rheometer: (**A**) Viscosity at 25°C/0.2 mm measuring gap, amplitude of 1% and frequency of 10 Hz (pure diazirine grafted polycaprolactone: CG^- and diazirine grafted polycaprolactone with bromothymol blue (CathoGlu; CG^+); (**B**) Statistical comparison of measured viscosities; (**C**) maximum storage modulus (G') at the total UVA absorbed dose of 10 J (CG^-) and 15 J (CG^+); (**D**) Representative gelation point (indicated by arrow) where G' = G'' (loss modulus) upon UVA activation (365 nm; 100 $mW.cm^{-2}$); **Statistical comparison of:** (**E**) Gelation points, expressed in UVA energy units (J); (**F**) Yield shear strength values measured from amplitude sweep (NSD-not statistically different; SD-statistically different; one-way ANOVA; $p<0.01$; $n = 3$).

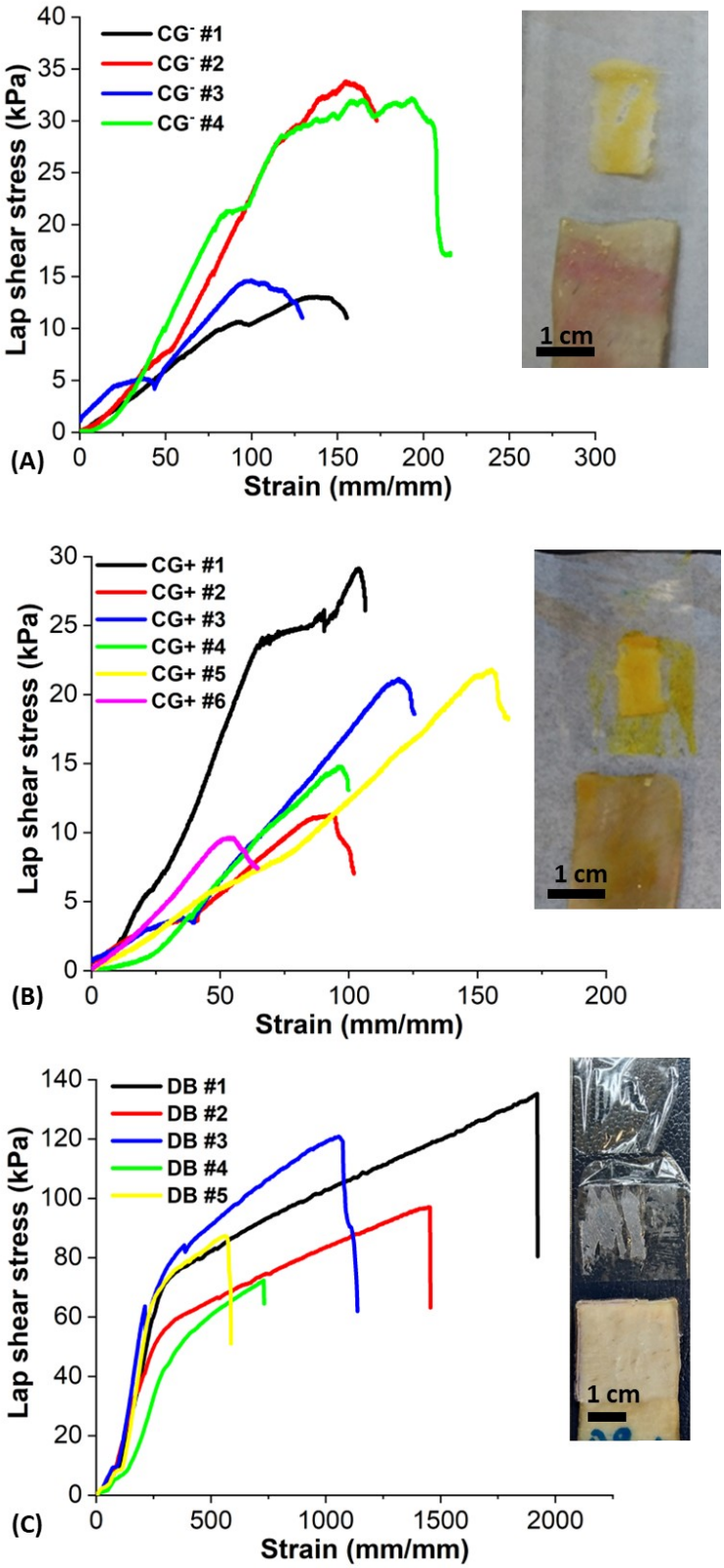

Figure S4. Lap shear stress vs strain curves recorded for skin tissue / adhesive / PET sheet interface: (**A**) CG^- after UVA activation at 10 J; (**B**) CG^+ after UVA activation at 15 J; (**C**) Dermabond™ (DB; control). Insets are showing corresponding, representative skin tissues, adhesive layers and PET sheets after shear failure.

Table S1. Sample preparation parameters for *in vitro* biocompatibility assessment of leachates in aqueous media (PBS and 0.1 NaOH). The liquid formulations (pure diazirine grafted polycaprolactone – CG^- and CathoGlu: diazirine grafted polycaprolactone with 1% bromothymol blue (CG^+) are placed in 6 well plates and subsequently crosslinked with UVA light (365 nm) for the total absorbed doses of 10 J (CG^-) and 15 J (CG^+) prior to incubation in aqueous medium for 72 h.

Sample	Mass (mg)	Total absorbed UVA energy (J.cm²)	Leaching medium	Leaching time (hours)	Citric acid 1M (uL)
CG⁻-10J-PBS	151	10	PBS	72	NA
CG⁻-10J-NaOH	151	10	NaOH 0.1M	72	145
CG⁻-0J-PBS	151	NA	PBS	72	NA
CG⁻-0J-NaOH	151	NA	NaOH 0.1M	72	150
CG⁺-15J-NaOH	152	15	NaOH 0.1M	72	212
CG⁺-15J-PBS	153	15	PBS	72	NA
CG⁺-0J-NaOH	152	NA	NaOH 0.1M	72	275
CG⁺-0J-PBS	151	NA	PBS	72	NA
BTB-Control	NA	NA	NA	NA	NA

1. *In vitro* and *in chemico* biocompatibility assessment of CathoGlu leachates: the experimental methodology

1.2 *In vitro* cell culture BlueScreen™ HC genotoxicity assay

The BlueScreen™ HC genotoxicity assay from Gentronix Ltd. uses a human-derived, p53-competent, TK6 cell line (GLuc-T01) to host a luminescence-based reporter system that exploits the proper regulation of the GADD45a gene. The genetically modified GLuc-T01 strain is maintained in RPMI 1640+ GlutaMAXTM medium supplemented with 10 % horse serum and 2.3% hygromycin B, 1.8 mM sodium pyruvate and 5.8% pen/strep antibiotics, maintained at at 37ºC in the presence of 5% CO2. The cells are harvested and resuspended in the proprietary complete assay medium and then distributed to the 96-well plates. A total volume of 75 µL (containing 2 x 106 cells/mL) is added per well. The leachate solutions are placed in the microplate and further diluted 2-fold with the complete assay medium

containing the GLuc-TO1 cells to produce the final plate containing 8 serial dilutions. The microplate is then incubated at 48 hours at 37ºC in the presence of 5% CO_2. The positive control compound (4-nitroquinoline 1-oxide) is prepared in 100% DMSO and then diluted 50x with sterile water to a final concentration of 0.5 µg/ml and 0.125 µg/ml. After incubation, the microplate is allowed to equilibrate at room temperature for 30 minutes. The injection solution for flash luminescence is prepared during this period, and the injector was primed with the injection solution. The microplate is then placed in the luminometer for reading: The luminometer is programmed to: (1) add 50 µl of the luciferase substrate to each well; (2) delay of 3 seconds before reading signal; and (3) integrate the luciferase activity for 5 seconds. Following luminescence data collection, 50 µl of the cell lysis reagent and DNA binding stain is added to each well of the microplate. The microplate is then incubated for 20 minutes at room temperature in the dark. After incubation, the fluorescence intensity data is collected using the fluorescein (485 excitation/535 emission filter). The genotoxicity threshold is set at a relative luminescence induction of 1.8, i.e. an 80% increase over the constitutive expression of GLuc. This statistically relevant threshold is set in excess of three times the standard deviation of the background fluorescence and that of the vehicle-treated controls. The genotoxicity result is based on the average brightness of the GLuc-T01 24e1 strain for the duplicate compound tests. A positive result was automatically given in a red box if 1 or more compound dilutions produced a luminescence induction greater than the 1.8 threshold. A negative result is assigned if none of the compound dilutions produced a luminescence induction greater than the 1.8 threshold.

1.2 *In vitro* cell culture sensitization KeratinoSens™ test (OECD-TG 442D)

The study is conducted following the test guidelines: OECD Test Guideline 442D *In Vitro* Skin Sensitization: ARE-Nrf2 Luciferase Test Method: KeratinoSens™. The transgenic cell line KeratinoSens™ with a stable insertion of the luciferase construct is maintained in Dulbecco's modified Eagle's medium (DMEM) containing Glutamax supplemented. with 9.1 % fetal bovine serum (FBS) and 500 µg/mL G418 at 37°C in the presence of 5% CO_2. The cells are harvested and resuspended in DMEM with 9.1% FBS without G418 and counted using a haemocytometer to obtain accurate cell density of cell stock solution. The cells are then seeded onto the 96-well plates. 125 µL containing 1 x 10^4 cells are seeded per well. After seeding, cells are grown for 24 hours in 96-well plates. The medium is then removed and replaced with fresh culture medium (150µL culture medium containing 1% serum,

without Geneticin) to which, 50 µL of leachates in aqueous media (12 serial dilutions in 2 repetitions) and controls are added. One well per plate is left empty (no cells and no treatment) to assess background values. The plates are then sealed with adhesive foils to prevent evaporation of volatile test items and cross-contamination between wells. The treated plates are then incubated for 48 h at 37ºC in the presence of 5% CO_2. After incubation, the exposure medium is aspirated from the white assay plates and discarded. The cells are washed once with PBS. 20 µL of 1x passive lysis buffer is added to each well, and the cells are incubated for 20 min at room temperature. The plates with the cell lysate are then placed in the luminometer for reading: The luminometer is programmed to: (1) add 50 µL of the luciferase substrate to each well; (2) delay of 1 second before reading signal; and (3) integrate the luciferase activity for 2 seconds. The positive sensitization results is assigned if the luciferase activity induction (I_{max}) measured is above the threshold of 1.5. Cinnamic aldehyde is used as a positive control in this study. Five concentrations of cinnamic aldehyde are used within each replicate in the KeratinoSens™ test method. A series of 5 master concentrations ranging from 0.4 to 6.4 mM are prepared in DMSO (from a 6.4 mM stock solution). The final concentration range of the positive control is from 4 µM to 64 µM.

1.3 *In chemico* skin sensitization: direct peptide reactivity assay (DPRA; OECD-442C)

The study is conducted following the test guidelines: OECD TG 442C: *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA). Stock solutions of the cysteine and the lysine containing synthetic peptides of purity higher than 85% are prepared freshly before incubation with the test chemical (leachate medium from crosslinked pure diazirine grafted PCLT (CG^-) and CathoGlu (CG^+). The final concentration of the cysteine peptide was 0.667 mM, 0.501 mg/mL in phosphate buffer at pH 7.5 ± 0.05 and lysine peptide was 0.667 mM, 0.518 mg/mL ammonium acetate buffer at in pH 10.2. Peptide standards were prepared in a solution of 20% acetonitrile: buffer using phosphate buffer (pH 7.5 ± 0.05) for the cysteine peptide and ammonium acetate buffer (pH 10.2 ± 0.05) for the lysine peptide. As described below, using serial dilution, standards of the peptide stock solution (0.667mM), 6 calibration solutions were prepared to cover the range from 0.534 to 0.0167mM. A blank of dilution buffer is also included in analysis. Cinnamic aldehyde at a concentration of 100 mM in acetonitrile is used as a positive control and is included in every assay run. HPLC system that includes HPLC, column, detector and autosampler (Shimadzu Prominence PDA System, Japan) is used in this assay. The column, Shim pack GIST-C18 with the dimension of 2.1 x

100 mm x 3µm is utilised. The vials are vortexed to mix and placed in HPLC autosampler (dark) at 25± 2.5°C for 24± 2 hours. HPLC analysis of the batch of samples started 24 hours after the test chemical is added to the peptide solution. Each test chemical iss analysed in triplicate for both peptides. The column temperature is maintained at 30°C. The incubation of samples in an autosampler is carried out at 25°C. Diode array detector in HPLC system detected the signal at 220 nm for the purpose of quantification of the concentration of chemicals. The positive sensitization is assigned for cysteine/lysine depletion >6.38%.

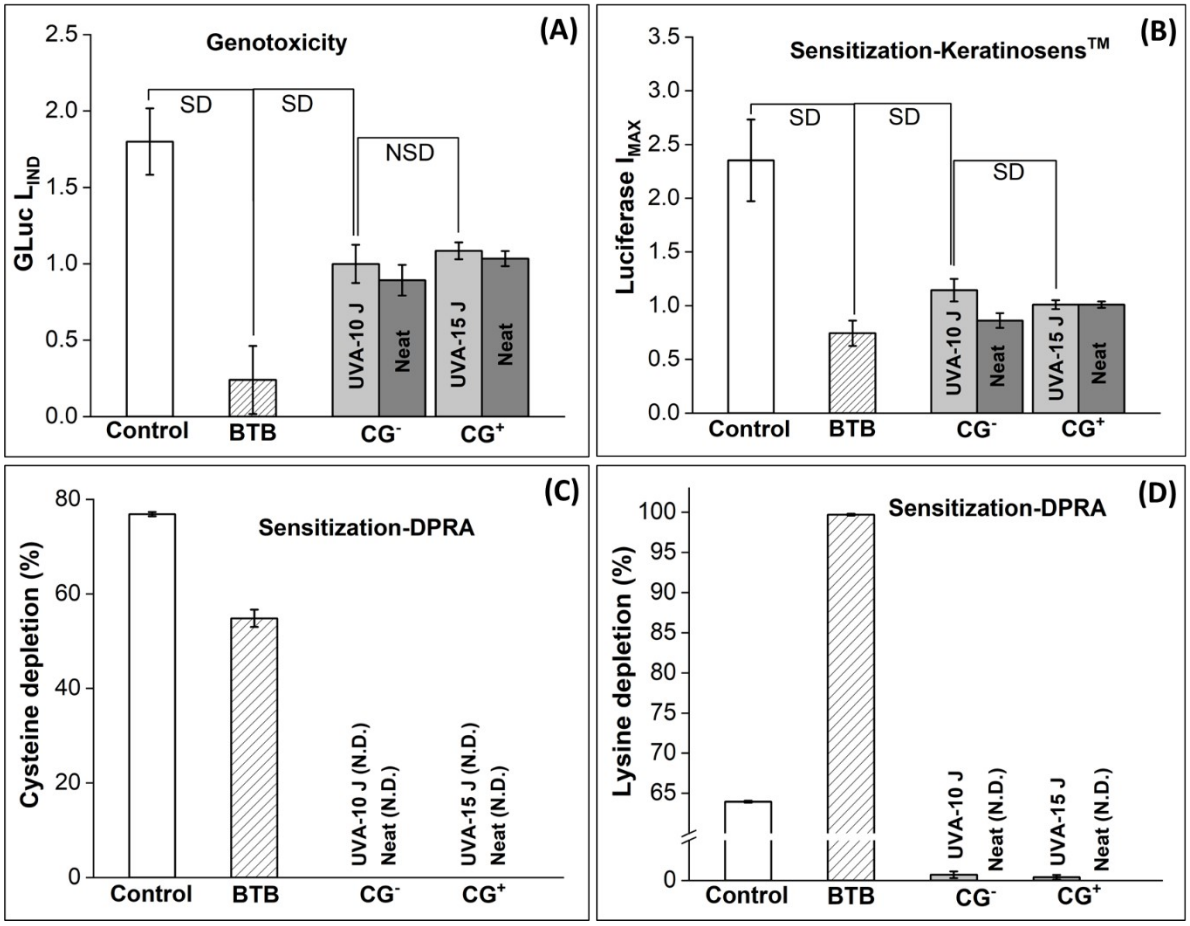

Figure S5. ***In vitro*** **biocompatibility of CathoGlu (CG^+) leachate in 0.1 M NaOH medium at physiological temperature after 72 h incubation, measured against the following controls: positive control (4-nitroquinoline 1-oxide), pure BTB pH indicator and diazirine grafted polycaprolactone (no BTB; CG^-):** (**A**) Genotoxicity BlueScreen HCTM results – CG^- / CG^+ "Neat" (liquid formulations without UVA activation), CG^- activated with UVA dose = 10 J; CG^+ activated with UVA dose = 15 J (applied in both genotoxicity and skin sensitization assays); (**B**) Skin sensitization assay KeratinoSens™ ARE-Nrf2 Luciferase (OECD 442D); (**C, D**) *In Chemico* direct peptide reactivity assay (DPRA; OECD 442C) with cysteine and lysine depletion, respectively. **Statistics: Genotoxicity** (**A**) standard deviation (10%) was calculated from average induction measured for control in 4 well plates and is applied to all measured samples; **Keratinosens™** (**B**) standard deviations are calculated for 2 repetitions of 12 dilutions; **DPRA** (**C, D**) standard deviations calculated from triplicate measurements (N.D. – Not detected; below detection limit; NSD – no statistical difference; SD – statistically different; one-way ANOVA; $p<0.05$).

Table S2. Draize dermal irritation scoring after catheter implantation on porcine ear observed daily (days 1 to 5) before sacrifice point at day-5; CathoGlu (CG^+), Dermabond™ (DB) and Micropore™ control (CT).

Sample	Erythema and Eschar Formation					Edema Formation				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
CG^+ 1	0	0	0	0	0	1	1	1	1	1
CG^+ 2	0	0	0	0	0	0	1	1	0	0
CG^+ 3	0	0	0	0	0	1	1	1	1	1
CG^+ 4	0	0	0	0	0	1	1	1	1	1
DB 1	0	0	0	0	0	1	2	2	2	1
DB 2	0	0	0	0	0	1	1	1	2	2
DB 3	0	1	0	0	0	1	1	2	1	1
DB 4	0	0	0	0	0	1	1	1	0	1
CT 1	0	0	0	0	0	0	0	1	1	1
CT 2	1	2	1	0	0	0	1	1	1	1
CT 3	0	1	1	1	0	0	0	2	1	1
CT 4	0	1	1	0	0	0	1	3	2	1

Table S3. Draize scoring system for: Erythema (redness of the skin caused by increased blood flow in superficial capillaries); Eschar (dead tissue and dried secretions from a skin wound following skin infection); and Edema (swelling caused by excess fluid trapped in tissue) [1].

Erythema and Eschar Formation	Edema Formation
No erythema **0**	No edema **0**
Very slight erythema (barely perceptible) **1**	Very slight edema (barely perceptible) **1**
Well-defined erythema **2**	Slight edema (edges of area well defined by definite raising) **2**
Moderate to severe erythema **3**	Moderate edema (raised approximately 1 mm) **3**
Severe erythema (beet- redness) to slight, eschar formation (injuries in depth) **4**	Severe edema (raised more than 1 mm and extending beyond the area of exposure) **4**

2. Histopathology analysis of dissected wound sites at catheter insertion point

The harvested tissues are fixed into cassettes and processed (Sakura VIP Tissue Processor) with alcohol, xylene and paraffin. Tissues are embedded into paraffin block and sectioned (rotary microtome) into 5 μm thick cross-sections at the wound site for staining (Haemotoxylin and Eosin; H&E by Leica Autostainer XL and Masson Trichrome; MT) and pathological evaluation of epidermis (wound) and dermis (blood vessel, disrupted by catheter insertion). The tissues are evaluated using ordinal scoring: No lesion-0; Minimal-1; Mild-2; Moderate-3; Marked-4; and Severe-5. The presence of scab area (μm^2 later converted to mm^2) and vascular wall thickness (μm) are measured using CellSens image software from images captured with DP71 Camera fitted on BX51 microscope (Olympus).

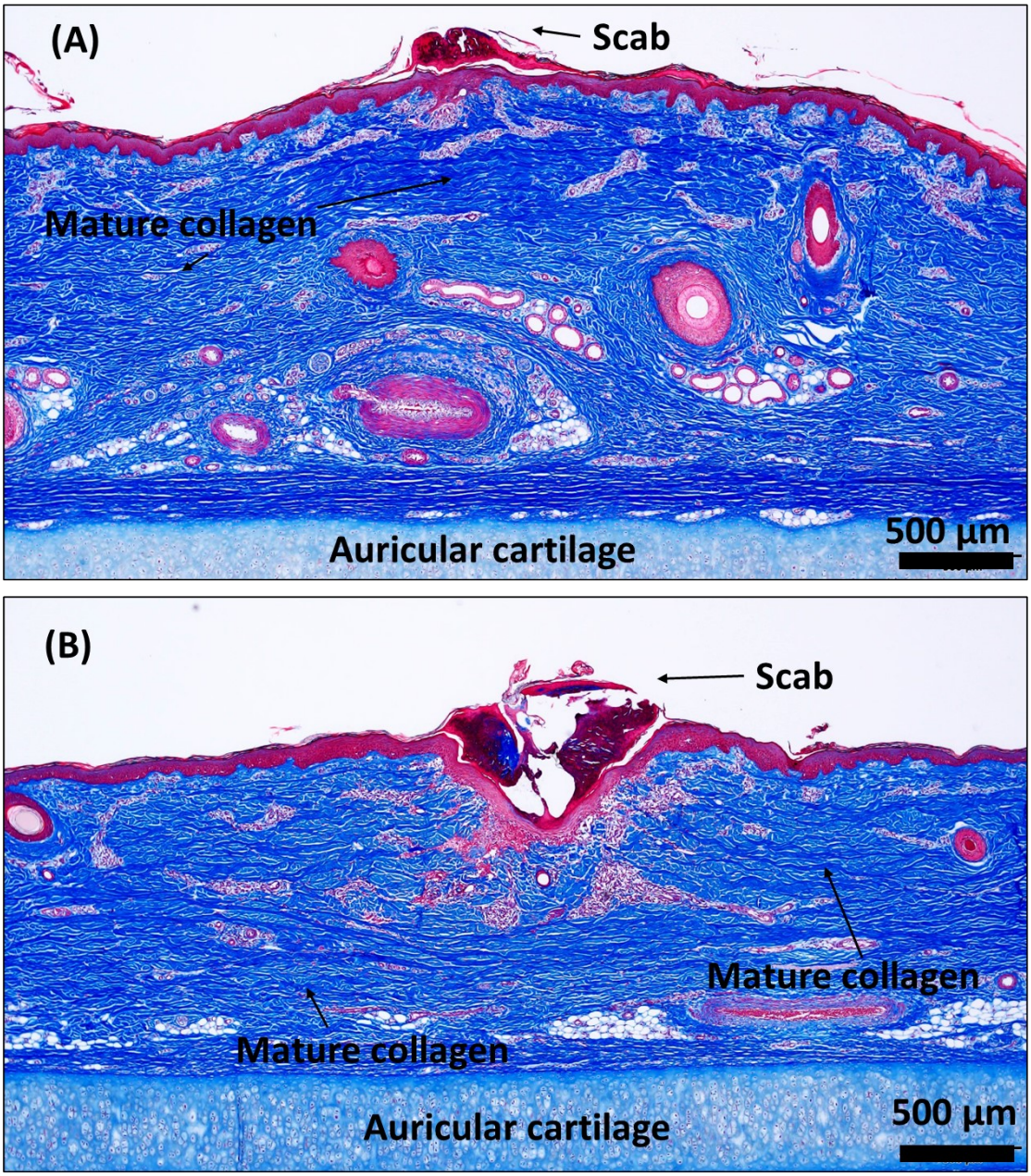

Figure S6. MT histology images of harvested tissue cross-sections at the catheter insertion sites (porcine ears) 5 days post-surgery indicating wound (scab) area and mature collagen in dermis area: (A) Dermabond™; **(B)** Micropore™.

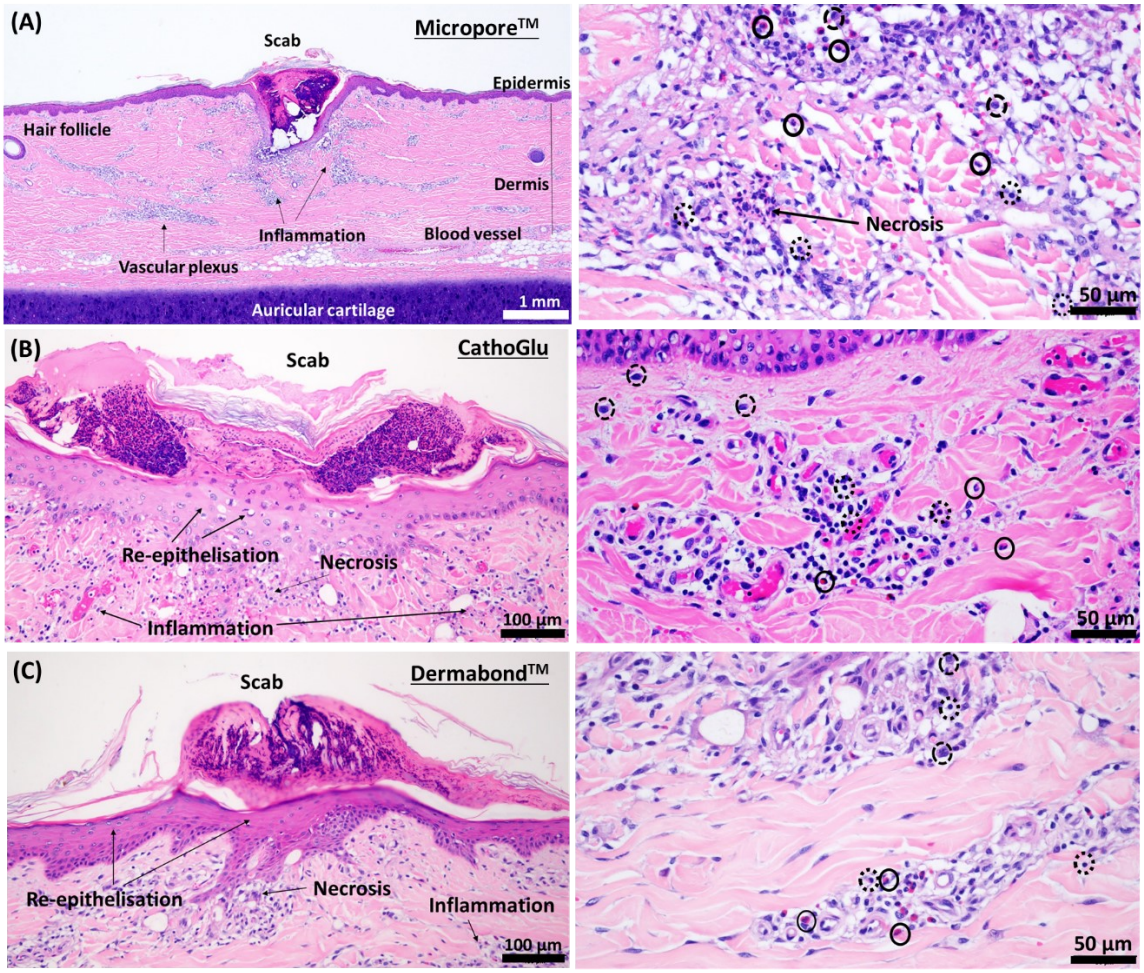

Figure S7. H&E histology images of harvested tissue cross-sections of scabs, formed at the catheter insertion sites (porcine ears) 5 days post-surgery: (A-C: Left) H&E histology images of harvested tissues cross-sections at wound site (scabs) with arrows indicating tissue structure and parameters for pathological assessment of catheter wound fixation by Micropore™, CathoGlu and Dermabond™, respectively; (**A-C: Right**) Representative, magnified H&E histology images indicating polymorpho-nuclear cells (full circles): macrophages (dashed circles) and lymphocytes, plasma cells (dotted circles) for overall pathological assessment (**Table S4** and **Figure 7H**) of Micropore™, CathoGlu and Dermabond™, respectively.

Table S4. Histopathology assessment (pathology score) of porcine ear skin wound site (scab) and blood vessel in dermis 5 days after catheter implantation sealed with: CathoGlu (CG^+; n = 4), Micropore™ control (CT; n = 4) and Dermabond™ (DB; n = 4).

MicD2:P33roscopic lesions	Ordinal score											
	1: minimal; 2: mild; 3: moderate; 4: marked; 5: severe											
	CG^+1	CG^+2	CG^+3	CG^+4	CT 1	CT 2	CT 3	CT 4	DB1	DB2	DB3	DB4
Epidermis												
Scab	0	3	2	1	4	2	3	2	0	1	1	2
Dermis												
Inflammation												
Polymorphonuclear	1	1	0	1	1	0	2	0	0	0	0	1
Lymphocytes	1	1	3	3	3	2	3	3	0	1	2	2
Plasma cells	0	0	2	2	2	2	2	2	0	0	2	2
Macrophages	1	1	1	1	2	1	1	1	0	0	1	1
Giant cells	0	0	0	0	1	0	0	0	0	0	0	0
Neovascularisation	2	0	3	0	0	0	0	0	0	0	0	0
Haemorrahges	0	0	0	0	0	0	0	2	0	0	0	0
Necrosis	2	1	1	1	2	1	2	2	0	1	0	2
Fibrosis	0	0	0	0	0	0	0	0	0	0	2	0
Total score	**7**	**7**	**12**	**9**	**15**	**8**	**13**	**12**	**0**	**3**	**8**	**10**
Mean total score (skin pathology)		**8.75**				**12.00**				**5.25**		
Blood vessel	CG^+1	CG^+2	CG^+3	CG^+4	CT 1	CT 2	CT 3	CT 4	DB 1	DB 2	DB 3	DB 4
Inflammation (perivascular)												
Polymorphonuclear	0	0	0	0	0	0	1	0	0	0	0	0
Lymphocytes	0	1	1	0	0	1	0	0	0	0	0	0
Plasma cells	0	0	0	0	0	2	1	1	0	0	2	0
Macrophages	0	0	0	0	0	0	0	0	0	0	0	0
Giant cells	0	0	0	0	0	0	0	0	0	0	0	0
Collagen deposition (perivascular)	0	0	0	0	0	0	0	0	0	0	2	0
Increased Collagen (Tunica media)	3	0	1	0	2	0	0	2	0	0	1	2
Medial hypertrophy	2	1	0	0	1	2	0	2	1	2	3	1
Thrombus- Occlusion	4	0	3	0	0	0	0	0	0	0	0	4
Thrombus- recanalisation	0	0	0	0	0	0	0	0	0	0	0	3

Table S5. Pathology scoring system for occlusion by thrombus.

Vascular occlusion by thrombus (%)	Severity	Score
0	0	Nil
1 to < 20	1	Minimal
20 to < 40	2	Mild
40 to < 60	3	Moderate
60 to < 80	4	Marked
80 to < 100	5	Severe

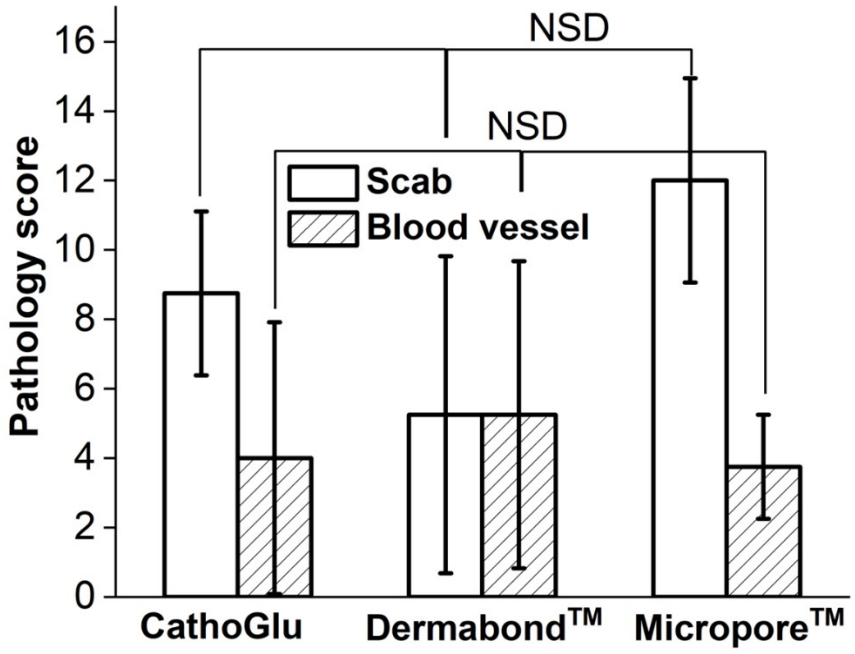

Figure S8. Statistical comparison of overall pathology score at scab (wound) and blood vessel sites (from Table S4) disrupted by catheter insertion and fixation with CathoGlu compared to Dermabond™ and Micropore™: Pathology assessment parameters: polymorphonuclear cells, lymphocytes, plasma cells, macrophages, giant cells neovascularisation, haemorrhages, necrosis and fibrosis (n = 4; NSD – no statistical difference; one-way ANOVA; $p<0.05$).

Table S6. Scab area on catheter insertion site (wound) and blood vessel wall thickness at catheter placement measured from H&E histology images, 5 days post-implantation: CathoGlu (CG^+), Micropore™ control (CT) and Dermabond™ (DB).

Animal ID	Scab area (mm^2)		Blood vessel wall thickness (µm)					
		Average ± Std.dev.	1	2	3	4	5	Average ± Std.dev.
CG^+ 1	NA	0.14 ± 0.07	58	63	76	61	70	70 ± 20
CG^+ 2	0.22585		152	67	59	56	73	
CG^+ 3	0.126536		66	80	46	52	55	
CG^+ 4	0.079607		80	80	88	83	94	
CT 1	0.335994	0.24 ± 0.07	100	65	91	141	80	80 ± 30
CT 2	0.187907		92	93	121	107	76	
CT 3	0.231023		55	48	38	34	44	
CT 4	0.19306		96	81	73	50	59	
DB 1	NA	0.09 ± 0.07	80	86	64	72	64	90 ± 30
DB 2	0.03681		136	70	82	63	101	
DB 3	0.064784		118	119	104	119	104	
DB 4	0.171942		64	49	155	45	40	
Skin (normal)	NA	NA	57	67	73	58	59	50 ± 20
Skin (normal)	NA	NA	37	69	34	39	34	

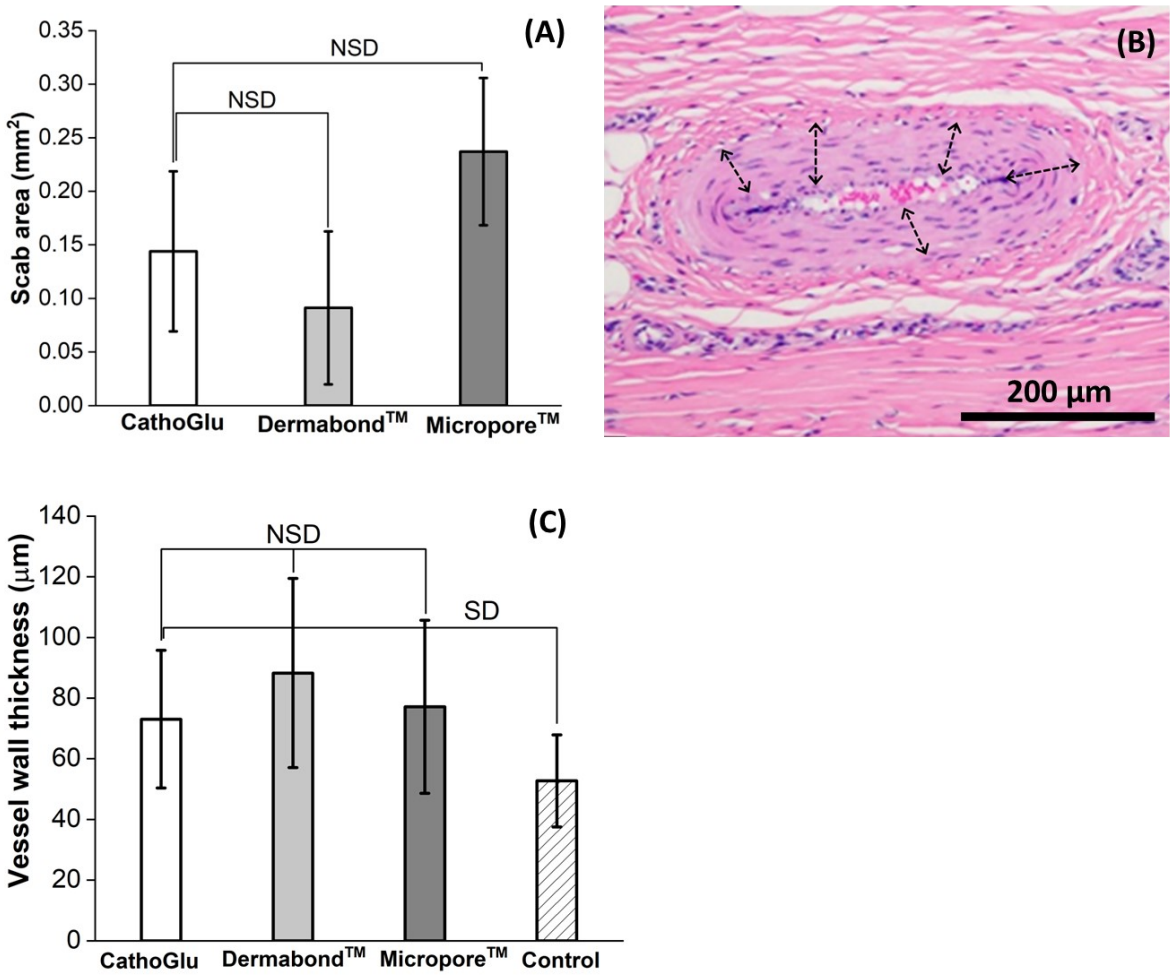

Figure S9. Statistical comparison of wound (scab) area (Table S6) and blood vessel wall thickness (Table S7) measured from H&E histological stain images 5 days post-surgery (porcine *in vivo* model) for CathoGlu catheter wound barrier, compared to Dermabond™ and Micropore™: (**A**) Scab area (mm^2) measured with microscope from H&E slides (n = 3-4); (**B**) Representative cross-section H&E image of healthy blood vessel used as control – double-headed arrows indicate the blood vessel thickness, measured at 5 random positions for observed vessel (bar = 200 µm); (**C**) Blood vessel thickness comparison (n = 20) for CathoGlu, Dermabond™, Micropore™ and healthy blood vessel (control) (SD – statistically different; NSD – no statistical difference; one-way ANOVA; $p<0.05$).

Size Exclusion Chromatography (SEC) of CG^- and CG^+ samples

CG^- and CG^+ samples (10 $mg.mL^{-1}$) dissolved in THF are irradiated for 100 s at 100 $mW.cm^{-2}$ (10J at 365 nm) and subsequently filtered with a 0.2 µm regenerated cellulose filter. A Shimadzu Prominence Ultra-Fast Liquid Chromatography (ULFC) system (Shimadzu, Japan) equipped with the Shimadzu RID-20A (Shimadzu, Japan) differential refractive index (RI), Shimadzu SPD-20A (Shimadzu, Japan) absorbance (UV-Vis), and miniDAWN TriStar Multi-Angle Light Scattering (MALS) detector (Wyatt Technologies, USA). The PLgel 5 µm column (Agilent, Singapore) was calibrated with an EasiVial PS-M pre-weighed calibration kit (Agilent, Singapore). The GPC was set to a mobile phase flow rate of 1 mL/min and each samples measured in triplicate. CG^- had a 960 ±10 kDa (PDI: 2.1± 0.1) and CG^+ had a 140 ±50 kDa (PDI: 4±2).

References:

1. M. Hemmati, A. Ghasemzadeh, M. Haji Malek-kheili, K. Khoshnevisan and M. K. Koohi, *Nanomedicine Research Journal*, 2016, **1**, 23-29.