Acceptor and donor one-pot voltage-activated adhesives

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2020

Tan, N. C. S. (2020). Acceptor and donor one-pot voltage-activated adhesives. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/146555>

<https://doi.org/10.32657/10356/146555>

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SINGAPORE

**ACCEPTOR AND DONOR ONE-POT VOLTAGE-
ACTIVATED ADHESIVES**

NIGEL TAN CHEW SHUN

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2020

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ACTIVATED ADHESIVES**

NIGEL TAN CHEW SHUN

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological
University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Nigel Tan Chew Shun

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

12th Aug 2020

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Date

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Assoc. Prof. Terry W.J. Steele

Authorship Attribution Statement

This thesis contains material from three papers published in the following peer-reviewed journal(s) where I was the first, joint first author, or joint author.

Chapter 4 is published as Lu Gan, **Nigel C. S. Tan**, Ankur Harish Shah, Richard D. Webster, Sher Li Gan, Terry W. J. Steele, Voltage-Activated Adhesion through Donor-Acceptor Dendrimers. *Macromolecules* 2018, *51* (17), 6661-6672. DOI: 10.1021/acs.macromol.8b01000

The contributions of the co-authors are as follows:

- I designed the study with Assoc. Prof. Terry W.J. Steele and Dr. Gan Lu.
- Dr. Gan Lu and I performed all the laboratory work related to the synthesis and samples characterization at the School of Materials Science and Engineering.
- Ankur Harish Shah performed COMSOL simulations.
- Sher Li Gan performed ^{19}F NMR experiments.
- Assoc. Prof. Richard D. Webster reviewed the manuscript.
- Dr. Gan Lu and I wrote the manuscript drafts which were edited and revised by all authors.

Chapter 5 is published as **Nigel C. S. Tan**, Animesh Ghosh, Terry W. J. Steele, Structure-Activity Relationships of Voltaglue Organic Blends. *Macromol. Rapid Commun.* **2020**, 2000188. DOI: 10.1002/marc.202000188

The contributions of the co-authors are as follows:

- I co-designed the study with Assoc. Prof. Terry W.J. Steele.
- I performed all the laboratory work related to the synthesis and samples characterization at the School of Materials Science and Engineering.
- Dr. Animesh Ghosh synthesized bromo-diazirine and reviewed the manuscript.
- I wrote the manuscript drafts which were edited and revised by all authors.

Chapter 6 is published as Lu Gan‡, **Nigel C. S. Tan‡**, Avi Gupta, Manisha Singh, Oleksandr Pokholenko, Animesh Ghosh, Zhonghan Zhang, Shuzhou Li, Terry W. J. Steele, Self curing and voltage activated catechol adhesives. *Chemical Communications* **2019**, 5, 10076 -10079. DOI: 10.1039/C9CC04166D

The contributions of the co-authors are as follows:

- I designed the study with Assoc. Prof. Terry W.J. Steele and Dr. Gan Lu.
- I performed all the laboratory work related to the synthesis and samples characterization at the School of Materials Science and Engineering.
- Avi Gupta performed UV/Vis spectroscopy experiments.
- Manisha Singh performed lap shear adhesion experiments.
- Dr. Oleksandr Pokholenko performed NMR analysis.
- Dr. Animesh Ghosh guided me in the synthesis of reduced catechol adhesive (reduced G5-DBA).
- Dr. Zhonghan Zhang and Asst Prof. Shuzhou Li performed density functional theory (DFT) calculations on the catechol and its tautomer intermediate.
- Dr. Gan Lu and I wrote the manuscript drafts which were edited and revised by all authors.

11th Aug 2020

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Date

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Nigel Tan Chew Shun

Abstract

Recent research has shown the voltage-activated adhesives are limited to water-based solvents. Non-conductive environment and high solvent resistance from the organic solvents are the main causes that lead to difficulty in curing of adhesive by voltage activation. On the contrary, curing the adhesive in water-based solvents like phosphate-buffered saline (PBS) results in no issue with voltage activation. However, water electrolysis occurs during voltage-curing. The generation of oxygen and hydrogen gases causes excessive foaming that compromises the structural properties of the adhesive. Thus, the advantages of replacing the water-based solvent with organic solvent are not limited to the adhesive application but can expand to other applications such as cosmetics and flexible implantable biosensors. Co-grafting the conductive additive ferrocene (Fc, donor) and the crosslinker diazirine (Dz, acceptor) onto the G5-PAMAM dendrimer in a biocompatible organic solvent (DMSO) can result in a donor-acceptor dendrimer. Fc is oxidized to Fc⁺ (donor) and provides a conductive hole network, while Dz is reduced to Dz⁻ (acceptor). Dz is oxidized to form carbenes, leading to intra/intermolecular crosslinking adhesion when voltage activation occurs. However, high donor-acceptor ratios co-grafted into one dendrimer leads to insolubility in organic solvents. Therefore, donor-acceptor blends overcome solubility limitations and provide faster throughput of structure-activity relationships with less synthetic investment. Despite these advantages, blend adhesives are constrained to intermolecular crosslinking adhesion upon voltage activation. In addition, the voltage activation concept of donor-acceptor pairs was not limited to ferrocene/diazirine designs. The concept was extended to donor-acceptor pairs containing catechol and a Schiff base, which crosslinked upon voltage activation. However, catechol adhesives are susceptible to air oxidation, which leads to short shelf-stability. Thus, further work may explore catechol variants, such as vanillin, to resolve the problem of shelf-stability while retaining donor-acceptor electrocuring. This thesis demonstrates the first proof-of-concept voltage-activated adhesive in organic solvents with donor-acceptor functional groups that can be initiated at low voltages (-1V to -5V, Ag/AgCl). This could contribute to the development of stimuli activated bioadhesives, drug delivery

methods, cosmetic coatings, flexible implantable biosensors, and industrial on-demand adhesives.

Lay Summary

This Ph.D. thesis presents a solution to overcome voltage-activated adhesives in organic solvents as the adhesive application **is** not limited to the aqueous formulation. The main hurdles that caused no curing from voltage activation in organic-based adhesives are high solvent resistance and poor current transmission. Electron donor-acceptor pairs grafted onto a dendrimer are introduced to improve the electron transduction within the adhesive. Upon applying low voltage, Fc oxidized to Fc⁺ (donor), providing conductive immobilized hole network, Dz reduced to Dz⁻ (acceptor), and Dz⁻ oxidized into carbenes, leading to crosslinking adhesion within the organic matrices. This process transits rapidly from a one-pot liquid adhesive to a viscoelastic solid form. Solubility in organic formulation becomes a problem when a high number of donor-acceptor ratios are co-grafted into one dendrimer. Hence, a donor-acceptor blend adhesive is developed for the studies of structure-activity relationships. This further demonstrated that voltage activation in an organic-based adhesive occurs in intra/intermolecular (cograft) and intermolecular distance (blend) between donor and acceptor. Lastly, using other donor and acceptor such as catechol and a Schiff base further confirmed the concept of donor-acceptor by voltage activation. One-pot voltage-activated catechol adhesive presents comparable adhesion strength with traditional two-part curing catechol adhesives. This will be the first one-pot voltage-activated adhesive that can be initiated at low voltage in water and biocompatible organic solvents which opens possibilities for clinical and industrial applications.

Acknowledgements

I would like to express the deepest gratitude to my project supervisor; Assoc. Prof Terry W.J. Steele for his continuous guidance inspiring conveyed motivation and elaborate scientific discussion in both research and findings. It would not have been possible without his supervision and regular follow up of the project development. His willingness to share his knowledge and experience allows me to accomplish what is presented in the Ph.D. thesis. Thank you, Prof Terry, for allowing me to work with you and nurture me.

I would also like to thank my mentors, Dr. Oleksandr Pokholenko, Dr. Gan Lu, and all the senior researchers, post-doc and fellow Ph.D. students in the lab, Dr. Zhou Yubin, Dr. Ivan Djordjevic, Dr. Richard O’Rorke, Dr. Himansu Sekhar Nanda, Dr. Ivan Solic, Dr. Animesh Ghosh, Dr. Richa Chaudhary, Dr. Ankur Shah, Dr. Raphael Ortiz, Dr. Elizabeth Ellis, Manisha Singh, Meyyappan Priyavadana, Elwin Ang and Gautama Wicaksono for their motivation and help. All of them are approachable and patient with me during the experiments as well as to ensure that the experiments were conducted in a safe and conducive environment when working together. I would like to specially thank Manisha Singh and Dr. Ivan Solic for their friendship and understanding in the lab. I would also like to thank all my NTU friends for their support in four years.

I would like to express my appreciation to my TAC members; Prof. Hu Xiao and Assoc. Prof Richard D. Webster for their guidance and excellent inputs in developing my Ph.D. project. I would like to thank all MSE staff for their support and understanding which made my Ph.D. experience great.

Lastly, I would like to thank my parents, siblings, family members (especially the McCann family), teachers, and friends (primary, secondary, polytechnic, army, NHG colleagues) for being constant support throughout my life. I would also like to thank my fiancée, Yan Hua, for her understanding, wise counsel, continual support, and encouragement, who is always there for me when I need her most.

This thesis is dedicated to the amazing woman in my life

Toh Yan Hua

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Abbreviations

PAMAM	Polyamidoamine , G5-PAMAM dendrimer
Dz	Diazirine
Fc	Chlorocarbonyl Ferrocene
Dz-	Diazirinyl radical
Fc+	Ferrocenium ion
G5-Dz	Diazirine grafted PAMAM dendrimer
G5-Fc	Ferrocene grafted PAMAM dendrimer
G5-Fc-Dz	Ferrocene cografterd PAMAM-g-diazirine
DBA	Dihydroxyl Benzaldehyde
G5-DBA	Donor-acceptor based Catechol grafted PAMAM
G5-Benz	Catechol-free benzaldehyde grafted PAMAM
G5-Vanillin / G5-Van	Vanillin grafted PAMAM
PLGA	Poly Lactic-co-Glycolic Acid
PET	Polyethylene terephthalate
PEG400	polyethylene glycol 400
SEC-GPC	Size exclusion chromatography
MALLS / MALS	Multiple angle laser light scattering
RI	Refractive index signal
dn/dc	Refractive index increment
G'	Storage modulus, Pa or kPa
G''	Loss modulus, Pa or kPa
G' _e	Effective Storage modulus, Pa or kPa
f	Frequency, Hz
ω	Angular frequency, rad/s
Tan δ / Tan δ	Damping factor/coefficient
η^*	Complex viscosity
G*	Complex modulus
PBS	Phosphate buffer saline
UV	Ultraviolet light
UVA	Near visible ultraviolet light (340-390 nm)
λ	Absorbance

ε	UV extinction coefficient
$v^{1/2}$	Square root of scan rate
L-DOPA	L-3, 4-dihydroxyphenylalanine
NaIO ₄	Sodium periodate
CV	Cyclic voltammetry
GC	Glassy carbon working electrode
Pt	Platinum counter electrode
Ag/AgCl	Silver/silver chloride reference electrode
Bu ₄ NPF ₆	tetrabutylammonium hexafluorophosphate
α -Al ₂ O ₃	α -Aluminium oxide polishing powder
DMSO	Dimethyl sulfoxide
DMF	Dimethylformamide
NMR	Nuclear magnetic resonance spectroscopy
¹ H NMR	Proton NMR
¹⁹ F NMR	Fluorine-19 NMR
¹³ C NMR	Carbon-13 NMR
UPO	Urea peroxide
BPO	Benzoyl peroxide
PU _s	Polyurethane
SARs	Structure-activity relationships
E_{pa}	oxidation potential
E_{pc}	reduction potential
ΔE_p	oxidation potential - reduction potential ($E_{pa}-E_{pc}$)
PPh ₃	Triphenylphosphine
CH ₂ Cl ₂	Dichloromethane
CBr ₄	Carbon tetrabromide
NaBH ₄	Sodium borohydride

Chapter 1

Introduction

Voltage-activated adhesives are constrained to water-based solvents and inapplicable to organic-based applications. Electrically insulating environments and high solvent resistance limit current conduction across these carbon-based matrices. Voltage-activated adhesives attempt to overcome this limitation with donor-acceptor dendrimers, a conceptual approach to achieve microamp to milliamp currents necessary for voltage activation of adhesives. Structure-activity relationships (SAR) centered around donor-acceptor solubility, grafting ratio, and balanced redox chemistries are identified. Hypothesis, objectives, and scope are summarized.

1.1 Problem Statement and Motivation

One-pot carbene-based adhesives in an aqueous formulation can crosslink either by UVA/photo-activation [1] or low voltage activation [2]. Additionally, the one-pot adhesive (PAMAM-g-diazirine aka G5-Dz) that undergoes UV activation is reported to reach a maximum storage modulus of 100kPa.[1] The two main components of this one-pot adhesive are a dendrimer backbone and carbene precursor. PAMAM dendrimers are soluble in aqueous and organic solvents and are commercially available in a variety of molar masses and surface chemistries. Previous investigations have identified G5-PAMAM-NH₂ variant has an optimal molar mass, with 128 surface primary amines available for surface modification. Diazirine serves as a carbene precursor, allowing stimuli dependent substrate and intermolecular covalent crosslinking. When curing using UV light [1, 3] or voltage at -1.6V [2], covalent crosslinking is facilitated by carbene insertion mechanisms.

Voltage-activated adhesives are envisioned to have several advantages over current methods of activation. For instance, unlike light and thermo-activated adhesives, voltage-activated adhesives can be cured on opaque substrates and thermally insulating materials, such as lumber and heat-sensitive plastics. The one-pot nature also avoids the limited pot life of two-part adhesive formulations. Therefore, a voltage-activated adhesive may address limitations encountered from UV/photo[4-6], thermal[7, 8], and chemical[9] activated adhesives.

However, the current voltage-activated G5-Dz adhesive operates only in aqueous formulations. No crosslinking is observed for the same adhesive in organic solvents, which is attributed to low current conduction. G5-Dz serves as a model system to develop the voltage-activated adhesive in an organic formulation, where aqueous formulations and their electrocured material properties are the working standard.

(G5-Dz) voltage-activated adhesives are current state-of-art, but several limitations are noted. First, they are constrained to voltage-curing in aqueous solvents from neutral to basic pH's. Water electrolysis occurs at the necessary

voltages for activation, which leads to foaming by oxygen and hydrogen gases. Oxidative intermediates at the anode are hypothesized to convert diaziridines to carbenes, which may induce or accelerate autooxidation. Last, the shear modulus of voltage-activated G5-Dz adhesive is 10-100x weaker than photo-activated formulations.

The use of organic solvents would avoid water electrolysis, which may increase the shear modulus matrices. However, electrical conduction needs to be overcome. A strategy is devised to increase current conduction by grafting internal conductive additives using a balanced redox concept. Grafting electron facilitators (e⁻ donor) and redox-activated crosslinkers (e⁻ acceptor) in close proximity through a dendrimer macromolecule complete the conceptual strategy. Sacrificial oxidants, degree of additives grafting, solvents, electrolytes, and other related dependent parameters are optimized to improve the crosslinking kinetics and storage modulus.

G5-Dz is utilized as a model system for the voltage-activated adhesive. The G5-PAMAM dendrimer has a macromolecule structure of 128 amino surface end groups. This spherical polymer backbone has little to no chain entanglement, which allows high solute formulations (e.g. 1:1 ratio of solute: solvent) to create viscous formulations requiring minimal solvents. Grafted diazirine (Dz) serves as a redox activated crosslinker upon voltage activation. Grafted ferrocene (Fc), also known as a metallocene, is a hole charge carrier known for its electron transduction properties. Ferrocene acts as an electron donor when it loses an electron and is oxidized to form ferrocenium. Conversely, diazirine acts as an electron acceptor when it gains an electron and is reduced to form a diazirinyl radical. Conductive pathways are hypothesized to contribute current transduction in organic-based adhesives when there is a presence of both donor and acceptor pairs attached to an immobilized network. The schematic illustration of the donor and acceptor redox reaction is shown in **Figure 1.1**.

Figure 1.1. Redox reaction of voltage-activated adhesive in organic formulation with ferrocene (Fc) as donor and diazirine (Dz) as acceptor

Structure-activity relationships for voltage-activated adhesives in an organic environment will also be determined. Donor-acceptor co-grafting versus blends are explored for faster throughput of structure-activity relationships with less synthetic investment. Alternative donor-acceptor pairs such as catechol and a Schiff base extend the concept to other known crosslinking strategies. These strategies eliminate gas evolution and may lower activation energy.

1.2 Research Hypothesis

Hypothesis 1 (Chapter 4): *Internal polymer grafting of donor–acceptor additives is required for electron transduction and contactless electrochemistry. One of the donor-acceptor additives must be electrochemically activated into a chemical crosslinker for voltage-activated adhesion.*

The G5-PAMAM dendrimer has a macromolecule structure of 128 amino surface end groups, allowing for the facile grafting of internal additives. When ferrocene

(Fc) is grafted onto G5-PAMAM, it will provide a conductive hole network, overcoming high solvent resistance in organic-based adhesives. The donor–acceptor theory from metal–organic frameworks (MOFs) inspires this design.

Hypothesis 2 (Chapter 4): *Organic miscible peroxides will replace electrolysis oxidants hypothesized for diaziridine oxidation and subsequent carbene formation and this increases crosslinking kinetics in organic solvents.*

The addition of sacrificial oxidants such as urea peroxide and benzoyl peroxide are expected to balance the redox reaction in organic environments and increase both crosslinking kinetics and storage modulus.

Hypothesis 3 (Chapter 5): *The correlation of Dz grafting percentage to adhesion strength is attributed to the number of carbenes, which is hypothesized to be the crosslinking moiety. Increasing Fc grafting is hypothesized to increase electron mobility by lowering the e^- tunnel length.*

With the introduction of the Fc and Dz co-graft adhesive, electron conduction occurs between intramolecular distance. However, high donor-acceptor ratios co-grafted into one dendrimer leads to solubility issues in organic solvents, preventing the identification of structure-activity relationships (SARs) of Fc and Dz contribution. Therefore, the development of the blend adhesive is expected to improve its solubility and explore Fc and Dz contributions.

Hypothesis 4 (Chapter 6): *Catechol (donor) is oxidized to o-quinone for chemical redox crosslinking. The concept of donor-acceptor voltage-activated adhesion will activate catechol to quinone if a suitable electron acceptor is established within close proximity.*

Catechol has been studied for quinone-mediated crosslinking and adhesion by two-part chemical curing. A new donor-acceptor pair is introduced, catechol and a Schiff base. Catechol can be oxidized to form o-quinone for crosslinking. It is

hypothesized that catechol is possible for voltage activation once the redox reaction is balanced using the donor-acceptor pairs concept.

1.3 Objectives and scope

According to the motivations and hypothesis that are stated above, the overall objective of this project is as follows:

“To determine the structure-activity relationships (SARs) of donor-acceptor voltage-activated adhesives in organic solvents using ferrocene diazirine co-graft and physical mixing ferrocene diazirine blend adhesives via electrorheology.”

To achieve the objective above, the following work in the Ph.D. thesis was carried out:

1. Ferrocene (15%) and diazirine (7%, 15%, and 30%) were synthesized onto one G5-PAMAM dendrimer forming co-graft adhesives. Mechanical properties of storage G' and loss G'' modulus and gelation time were evaluated.
2. The adhesion strength of co-graft adhesives was evaluated against tack adhesion tests in electrorheometry.
3. Sacrificial oxidants such as urea peroxide and benzoyl peroxide were added to G5-Fc-Dz co-graft adhesive to improve crosslinking kinetics.
4. 7%, 15%, and 22% of G5-PAMAM-grafted ferrocene were synthesized, 7%, 15%, and 30% G5-PAMAM-grafted diazirine were synthesized. Both dendrimers were blended to conduct electrorheology. The mechanical properties of storage G' and loss G'' modulus and gelation time were evaluated to correlated to Fc and Dz contributions.
5. Lap shear adhesion strength was assessed against collagen film substrates and compared between ferrocene diazirine co-graft and ferrocene diazirine blend adhesives.

6. Other donor-acceptor pair which is electrochemically activated below water electrolysis such as catechol and a Schiff base, were synthesized with the G5-PAMAM dendrimer and underwent electrorheometry analysis. The structure-activity relationship of the voltage-activated one-pot catechol adhesives was evaluated.
7. Lap shear adhesion strength of the catechol adhesives was assessed against collagen film substrates and compared with traditional two-part curing catechol adhesives.

1.4 Dissertation Overview

This thesis consists of the following seven chapters:

Chapter 1 states the current limitation of voltage-activated adhesives and the motivation for this research. Hypotheses, thesis scope, dissertation overview, and the innovative outcomes are listed.

Chapter 2 presents the current progress of voltage-activated adhesives, with focus on creating a conductive system in organic solvents and their mechanical properties. Previous literature gives a brief review of organic adhesives and the design of organic voltage-activated adhesives.

Chapter 3 discusses the strategies and rationale for the materials and methods selection, including choice of polymer backbones, charge carriers, and crosslinker to form the donor-acceptor relationship. The experimental design of the synthesis, characterization, and performance evaluation methods is described.

Chapter 4 introduces the donor-acceptor co-graft developed for voltage-activated adhesives in organic solvents. The material properties are dependent on diazirine grafting ratios with a fixed grafting ratio of ferrocene. The addition of sacrificial precursors such as urea peroxide and benzoyl peroxide are hypothesized to improve the crosslinking kinetics and storage modulus. All are measured in real-

time electrorheometry analysis. The adhesion strength is evaluated against a tack adhesion test in electrorheometry.

Chapter 5 addresses the detailed structure-activity relationship for donor-acceptor blends using real-time electrorheometry analysis. The crosslinking kinetics and mechanical properties on three different grafting ratios of ferrocene and three different grafting ratios of diazirine are assessed. The adhesion strengths are evaluated using the substrates of collagen films.

Chapter 6 summarizes that the voltage activation concept of donor-acceptor pairs is not constrained to ferrocene and diazirine. Other donor-acceptor pairs such as catechol and a Schiff base, which are electrochemically activated below water electrolysis are assessed using real-time electrorheometry analysis. The adhesion strength of the catechol adhesives is evaluated against the substrates of collagen films.

Chapter 7: summarizes the results of the thesis and outlines recommendations for future research.

1.5 Findings and Outcomes/Originality

Several outcomes were achieved in this thesis.

1. High solvent resistance and poor conductivity in organic adhesive formulations were overcome by introducing an immobilized hole network of ferrocene grafted onto the G5-PAMAM macromolecule dendrimer.
2. The solubility issue of the donor-acceptor co-graft adhesive is resolved by developing the donor-acceptor blend adhesive and this has provided faster throughput of structure-activity relationships.
3. Increasing Fc (donor) contributes to faster gelation time while increasing Dz (acceptor) contributes to better adhesion strength.

4. Electron conduction occurs in both inter (blend) and intra/intermolecular distance (cograft) between donor and acceptor.
5. Adding sacrificial oxidants such as urea peroxide and benzoyl peroxide shows no effect in the crosslinking kinetics and storage modulus.
6. The concept of donor-acceptor by voltage activation works for other donor-acceptor pairs (catechol-Schiff base). Not only it achieved faster gelation time and better adhesion strength with no gas emission, but also voltage activation occurs below water electrolysis.

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Chapter 2

Literature Review

The research gap of voltage-activated adhesives is addressed with respect to the hypotheses in preceding chapters – which includes the background of structural adhesives and stimuli-activated adhesives, poor conductivity of voltage activation in organic formulations, and inspiration of donor-acceptor theory from metal-organic frameworks to voltage-activated adhesives.

2.1 Background: Conventional adhesives are organic-based, and they are activated by chemical, thermal, and photocuring.

For the past fifty years, the methods of adhesive activation have followed the same basic methods. These methods revolve around two-part chemical, thermal, or photo-activation. Chemical curing adhesive is usually a two-part adhesive (liquid monomer and hardener) which requires mixing to induce curing. Two-part chemical curing adhesives currently display the strongest adhesion strengths. Epoxy thermoset resins have young's moduli comparable to some metals, giving them the designation of structural adhesive. However, chemical curing adhesives have several drawbacks. This includes the duration of chemical curing with no end-user instant activation, the adhesives have nominal thermal conductivity typical of polymer-based resins (e.g. epoxy at $0.19 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$, polyurethane at $0.29 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ [1]), brittle failure properties lacking ductility or elongation and susceptible to ageing due to presence of moisture and UV light [2]. To impart some level of end-user control, formulations have incorporated event-or stimuli-activated initiation. Some examples of stimuli activated initiation include thermal activation or photo-activation.

Thermal curing adhesive such as moisture curing polyurethanes (PUs) has the advantage of able to seal to temperature-insensitive substrates but required hour-long curing times. The most common drawback of thermal curing adhesive is cannot be utilized on heat-sensitive materials, such as plastics [3]. Other adhesive like cyanoacrylate adhesive can adhere well on rubber and most plastics but it is brittle when cured and has limited hydrolytic and thermal stability up to 80°C . On the other hand, photocuring adhesive provides adhesion strength upon curing but catalyst such as photoinitiator is required and only applied on the transparent substrate. Most of the substrates in manufacturing industries are not transparent; therefore it will only have niche applications.

Chemical curing and thermal curing adhesive are widely used for many industrial applications and they are usually in organic-based formulation—these organic

thermoset materials have some of the most resilient material properties, as they are especially resistant to solvents and organic acids and bases. Some examples of organic thermosets are epoxies, which serve as the matrix for many high-performance composites. This requires a combination of low viscosity (<1 Pa.s) before curing and high-crosslinking density after activation ($>90\%$ functional group activation).

With the disadvantages from chemical, thermal, and photocuring adhesives, the project aims to design one-pot voltage-activated adhesive which allows end-user to activate a liquid-based adhesive to a viscoelastic solid and manipulate the desired adhesion strength that can overcome two-part chemical and thermal curing adhesives. The advantage of introducing voltage-activated adhesive is that it can be applicable to heat-sensitive materials and opaque substrates. Therefore, if voltage-activated adhesive can be designed to transition from a liquid to solid curing via ampere-dependent stimulus, current problems from various curing methods may be overcome and provide an additional option for end-users. Previous technologies have attempted to create coatings via current stimulation, known as electrografting.

One method to adhere to an organic layer onto conductive substrates (e.g. metal, carbon, dielectric polymer) is by electrografting. This method involved an electron transfer between the to-be modified substrate as well as the reagent. Upon voltage activation, grafting between the conductive substrate and organic layer takes place which serves as a starting point for voltage-activated adhesive designs. Electrografting has been shown to prevent metal corrosion via oxidative and reductive crosslinking [4], [5]. Conductivity of electrografting was enhanced with ferrocene grafting onto an amino group, which may serve as a hole-carrier [6]. However, limitations of the electrografting mechanisms prevent its incorporation as adhesives. Electrografting allows surface grafting, but no further reactions beyond a few nanometers occur into the bulk. Voltage-activated adhesive attempts to crosslink a resin between two inert substrates that is on the order of 0.1 to 1 mm thick.

Polyamidoamine (PAMAM)-g-diazirine serves as a model system to develop the voltage-activated adhesive. PAMAM-g-diazirine provides a dendrimer structure with 128 surface end groups for crosslinker such as diazirine to graft on it and storage modulus comparable to gelatin (300Pa-1kPa) [7]. PAMAM dendrimer acts as a polymer backbone in the adhesive which is not conductive in nature for voltage curing. With the grafting of redox-active crosslinker on the dendrimer in an aqueous formulation, voltage curing is possible. This PAMAM-g-diazirine adhesive can be electrocured in an aqueous solvent like phosphate-buffered saline (PBS) [8]. In this case, PAMAM acts as a polymer backbone while diazirine forms into diaziridine, followed by oxidized to carbenes which are hypothesized to promote crosslinking. However, the current on-demand voltage-activated adhesive is limited to an aqueous salt solution. In the market, common adhesives such as epoxies and acrylates are organic-based formulations. To broaden the scope of voltage-activated adhesive application, it is feasible to translate the model system of PAMAM-g-diazirine in the aqueous formulation to organic formulation. However, this voltage-activated adhesive in organic formulation displays no curing upon voltage activation due to high solvent resistance and poor conductivity. In addition, several problems encountered for voltage-activated adhesive in an aqueous formulation which can be overcome if the formulation is changed to organic-based. One example is water electrolysis which produces hydrogen and oxygen will occur when voltage activation is > -1.6 V and it may compromise the adhesion strength as gases create foam within an adhesive matrix. If this system could be activated within an organic formulation, it would prevent water electrolysis. As such, only inert nitrogen gas will be a by-product generated from the activation process. However, N_2 gas will produce foam during voltage curing, limiting the crosslinking density. Diazirine is a carbene precursor which has been utilized as an adhesives crosslinker for various surface modifications upon photo-activation [9, 10] and protein, carbohydrate, and enzyme modification for other biological uses [11-13]. Therefore, the motivation of one-pot voltage-activated adhesive in an organic formulation can widen its application and eliminating the drawbacks of chemical, thermal, and photocuring adhesives.

2.2 Limited methods of voltage/current crosslinking: Diazirine based adhesive crosslinking in an aqueous formulation

Supposition 1: *PAMAM-g-diazirine displays no crosslinking in acetonitrile/electrolyte solvents, but this can be overcome by incorporating conductive donor/acceptor pairs.*

Feng et al. synthesized PAMAM-g-diazirine adhesive which can either activated by UV dose or low voltage electricity in the aqueous formulation [8, 14]. These examples show that tunable mechanical and adhesion strength, as well as viscoelasticity and its modulus, can be achieved in the initiation and propagation stage from either UV dose which converted diazirine into carbenes for UV crosslinking (**Figure 2.1**) or low voltage electricity (only in aqueous formulation) by using diazirine that converted diaziridine, followed by oxidized to carbenes which hypothesized to promote voltage crosslinking (**Figure 2.2**). Due to the tunable mechanical performance of the diazirine-based adhesives in the aqueous formulation [12], the research aims to achieve adhesion strength in kPa range. UV activated adhesive is limited to transparent substrates and visible surfaces [15]. Therefore, the development of low voltage-activated adhesive with tunable material properties aims to give an alternative to overcome the limitations of UV activated adhesive by providing gradient curing within the adhesive matrix and to cure using transparent substrates and visible surfaces[8].

Figure 2.1. a) Diazirine chemical mechanism after light ($h\nu$) or heat (Δ) activation, b) Carbene crosslinking after UV activation

There are little research and development of adhesive with low voltage activation for organic formulation. The voltage-activated adhesive in organic formulation displays no curing upon voltage activation due to high solvent resistance and poor conductivity. The choice of redox-active crosslinker is diazirine. In the UV curing system, carbenes derived from diazirine is the main mechanism for surface binding and crosslinking of polymer (**Figure 2.1**). On the other hand, the voltage-activated intermediates in PAMAM-g-diazirine formulation consist of diazirinyl radicals/diaziridine produced at the cathode (which has been reduced) while the hypothesized carbenes are produced at the anode (which has been oxidized). The half-reaction of diazirinyl radicals/diaziridine and hypothesized carbenes are illustrated in **Figure 2.2**.

The voltage curing of the PAMAM-g-diazirine adhesive is involved not just only on the surfaces of the anode and cathode but also undergoes gradient crosslinking within the bulk polymer medium [16]. The rheology and adhesive results from Ping et al presents that the crosslinking is involved after voltage activation to increase the mechanical performance of the PAMAM-g-diazirine [8]. However, the PAMAM-g-diazirine adhesive is constrained to voltage-activated in an aqueous formulation.

The diazirine is attached to the intramolecular surface end groups of PAMAM to give crosslinking. Ping et al have reported that the higher amount of diazirine conjugated onto the immobilized PAMAM dendrimer, there is current and crosslinking kinetic increases by voltage curing as it is due to more radicals involved [8]. It may be due to more diazirine grafting onto PAMAM and result in obtaining the shorter intermolecular distance between the diazirines. This shows that when a low voltage potential is applied, diazirine is reduced to diazirinyl radicals/diaziridine and allows electron hopping and tunneling from the cathode side of the electrode. Then the diaziridine oxidized (illustration shown in **Figure 2.2**) and formed hypothesized carbene which allows non-specifically substrate and intermolecular crosslinking. In addition, PAMAM dendrimer has a molecular weight of 28 kDa which is a suitable polymer backbone for crosslinker grafting so that radicals can transport electrons in sensor-based applications efficiently [17, 18]. However, the voltage cured

PAMAM-g-diazirine adhesive is achieved in an aqueous formulation only. Most adhesive formulations available in the industries are in organic formulations (e.g. Epoxies and acrylates).

The research interest is to make a conductive environment for voltage-activated adhesive in the organic formulation, as such by utilizing a high molecular weight macromolecule (e.g. PAMAM) to immobilize hole charge carrier and redox-active crosslinker (e.g. diazirine, Dz). The presence of hole charge carriers can form donor and acceptor pairs in the organic environments so that conductive pathways can be achieved. Organometallic compounds such as metallocenes are known for their for-hole charge carrier attributes, e.g ferrocene.

On the other hand, diazirine which grafted onto the dendrimer forms hypothesized carbene when activated by the voltage which leads to crosslinking. Thus, it creates the system of immobilized donor and acceptor network where metallocene (donor) oxidized into metallocene⁺ and Dz (acceptor) reduced into Dz⁻. To overcome high solvent resistance of adhesive in organic formulations, *hypothesis 1* creates an immobilized conductive environment by donor/acceptor grafting and it is inspired by donor-acceptor theory from metal-organic frameworks (MOFs). Metallocene/Metallocene⁺ acts as a hole transfer carrier/electron donor while diazirine accepts electron and reduced to diazirinyl radicals/diaziridine, followed by hypothesized carbene which leads to crosslinking and an increase in storage modulus. Hence, using metallocene as conductive charge carrier (donor) and diazirine as redox-active crosslinker (acceptor) to graft onto the polymer can have the potential to bridge this gap and allow adhesives to achieve adhesive crosslinking in the organic formulation. Furthermore, on-demand diazirine-based one-pot voltage-activated adhesive has the possibility to broaden its application to flexible biosensors, cosmetics, and opaque substrates.

Figure 2.2. Half reactions of diazirinyl radicals/diaziridine and hypothesized carbenes in aqueous formulation

2.3 Voltage-activated adhesive in organic formulation require oxidants to balance the redox reaction to improve its mechanical performance

Supposition 2: *Sacrificial oxidant will balance the redox reaction of voltage-activated adhesive in the organic formulation to increase crosslinking kinetics*

The advantage of voltage curing PAMAM-g-diazirine in an aqueous formulation is that it can control the electrochemical activated functional groups by simply turning on and off the voltage supply, thus end-users can manipulate adhesion strength up to tens of kPa. If adhesives can be activated by low voltage, one interesting application will be to replace lead-based solders using thermoset voltage-activated adhesive as bonding medium on printed circuit board (PCB) to solve environmental issues which have been discussed by R. Gomatam and K.L. Mittal [19]. Voltage-activated adhesives may be available for end-users who encounter the drawbacks from chemical, thermal, and photocuring adhesives.

There are limitations of PAMAM-g-diazirine adhesive in PBS solution when it comes to voltage curing system. When diazirine is reduced to diazirinyl radicals/diaziridine at the cathode side, water helps to balance the redox reaction at the anode side. The half-reaction of water electrolysis oxidation and diazirine reduction are shown in **Figure 2.3**.

Figure 2.3. Half reaction of water electrolysis oxidation and diazine reduction

However, the model system of the adhesive, PAMAM-g-diazirine, only has diazine involved in the redox reaction, thus when diazine reduced to diaziriny radicals followed by further reduced into diaziridine, it will then oxidized into hypothesized carbenes to balance the redox reaction which eventually causes nitrogen gas to be generated (refer to **Figure 2.2**). As such, the rate of crosslinking is limited as the adhesive only voltage cured starting from the cathode side and slowly cured towards the anode side with a gradient [16]. PAMAM-g-diazirine in organic formulation does not have water to balance the redox reaction in order to produce hypothesized carbenes for voltage crosslinking. Hence, to balance the redox reaction and to improve the rate of crosslinking, *hypothesis 2* can balance the redox reaction by addition of sacrificial oxidants precursor such as peroxides or nitrenes (e.g. oxidants like nitrene and benzoyl peroxide which are shown in **Figure 2.4**) that responsible for oxidation instead of diaziriny radicals/diaziridine to be oxidized [20]. As such, it is hypothesized to improve crosslinking kinetic and it can be determined by rheology test via voltage activation method.

When diaziriny radicals/diaziridine is oxidized into carbenes, the mechanical properties are affected by gas evolution (H_2 , O_2 , N_2). In previous research, PAMAM-g-diazirine adhesive can dissolve in the aqueous formulation without compromising its mechanical property when photocured. Hence, photocuring results in a higher storage modulus G' (100kPa) compared to voltage curing

adhesive (1kPa). As for voltage activation, PAMAM-g-diazirine in PBS solution has water to balance to the redox reaction but voltage activation $> -1.6\text{V}$ leads to water electrolysis may compromise the mechanical properties of adhesive. To prevent water electrolysis to occur, it can be replaced by aprotic organic solvents such as DMSO. However, the organic solvent has high solvent resistance for voltage curing which leads to ions migration, thus causing the adhesive in the organic solvent to have poor conductivity for voltage activation.

a

b

Figure 2.4. Using sacrificial oxidants to balance the redox reaction, in this case, a) nitrene and b) benzoyl peroxide

At the time being, there is no finding of voltage-activated adhesive that can be cured in organic solvent formulation due to its non-conductive property in nature. Replacing aqueous formulation with the organic formulation that can eliminate water electrolysis and hypothesized to lead the range of mechanical properties equal to photocuring.

2.4 Introducing donor-acceptor pairs to create conductive environment in organic-based voltage-activated adhesive

Supposition 3: *Improve conductivity in an organic environment by adapting the concept of metal-organic frameworks with donor-acceptor pair.*

Metal-organic frameworks (MOF) consists of compounds such as metal ions and organic ligands. They are often porous and high surface area materials that are usually used for many applications such as gas storage [21-23] and separation [24]. MOF is a coordination polymer that has an organometallic polymer structure with metal ions in the center linking with organic ligands at the side. It has coordination repeating and can be extended to 1, 2, 3 dimensions which illustration is shown in **Figure 2.5** [25]. MOF has low electrical conductivity which limits their applications for fuel cells and supercapacitors [26-32].

According to the conductivity equation ($\sigma = e(\mu_e\eta_e + \mu_h\eta_h)$), conductivity (σ) is possible when the charge density (η) is high as well as the electrons (e) and holes (h) mobility (μ) are high too. Since MOF has both metal ions and organic ligands, it can be the source of free charge carriers which can improve the conductivity of the materials. It is because metal ions have unpaired electrons that consist of high electrons or holes energy. These unpaired electrons in the conductive band are all free charge carriers which result in high charge density and it leads to an increase in electrical conductivity ($>100 \text{ S.cm}^{-1}$) [33]. While on the other hand, semi-conductor and insulators have fermi level which has a bandgap in between the conduction and valence band. Thus, thermal activation is required for a semi-conductor to move electrons from the valence band to the conduction band.

As there is a porous structure within the MOF, it can offer by incorporating conductive charge carrier to create a conductive pathway which MOF is lack of in the beginning. By adding redox-active dopants within the MOF pores, it can provide charge transfer interactions [34]. Thus, charge transfer interactions provide the donor and acceptor pairs between the MOF and the dopants which results it to have conductive pathway. In this case, the MOF consists of metal ions and organic ligands, metal ions have unpaired electrons and tend to oxidize.

Metal ions are the donor. On the other hand, introduce redox-active dopants acts as an acceptor to create a charge-transfer interaction. Electron hopping occurs when charge-transfer interactions, therefore conductivity increases. Hence, MOF can achieve from a non-conductive network to a conductive network by introducing dopants. However, the drawback of adding dopants in MOF may lead to a reduction of pores and limit the applications such as gas storage, separation and it is utilized in solid materials.

Metal Organic Framework in One Dimension

M = Metal Ions

L = Organic Ligand

Figure 2.5. One example of metal-organic frameworks (one dimension) where M is metal ions while L is organic ligands [25]

By adopting the concept of MOF from a non-conductive network and adding dopants to make it conductive, the donor-acceptor theory can be translated and applied to the proposal of voltage-activated adhesive in the organic formulation. The adhesive has a redox-active crosslinker which can be crosslinked when there is an occurrence of a redox reaction, current and voltage applied. However, the organic environment does not allow current to pass through due to high solvent resistance. Thus, making the adhesive in organic solvent unable to cure using voltage. Since the organic solvent is not conductive in nature, it is similar to MOF which is not conductive to begin with. A redox-active dopant can be introduced into the adhesive and it will provide charge-transfer interactions with the redox-active crosslinker. Metallocene such as ferrocene ($\text{Fe}(\text{C}_5\text{H}_5)_2$) is an organometallic compound with good redox reversible properties and oxidized at low potential. It can generate stable redox form and form an electron/hole mobility charge network which is an ideal candidate as a dopant to improve the adhesive conductivity. As ferrocene (Fc) has reversible redox properties, it can act as a donor when oxidized and an acceptor when reduced. On the other hand,

the redox-active crosslinker will accept an electron from the ferrocene and have charge transfer interaction between each other, thus electron hopping occurs, and voltage-activated adhesive can be possible in an organic solvent by electron transduction between the donor and acceptor pairs.

Ion migration upon voltage activation resulted to high solvent resistance which leads to a double layer. At the surface of the positive charged particle, it will tend to attract negative charged ions. This is due to opposite charges attract and that negative charged ions formed a double layer around the positive charged particle. At the outer part of the double layer is surrounded with a diffused layer. This diffused layer has a mixture of positive and negative charged ions that come from the solvent or solution. When voltage is applied, the ions of the solvent will tend to migrate depending on the charges of the double layer. Since the double layer is negative charged, positive ions will migrate to the negative charged double layer. As time goes by, the ions will be segregated accordingly and no free ions available in the solvent for current flows. Thus, resistance is built up by the solvent.

In order to reduce the solvent resistance and achieve current flows from voltage curing, larger molecules are introduced in the solvent. Ferrocene is then introduced for electron transduction. Even though it is a larger molecule, ferrocene will tend to transfer electrons and form positive charged ferrocene, which is called ferrocenium ions. Thus, ion migration will still be occurring in a matter of time and solvent resistance will build up. Therefore, ferrocene is grafted on the macromolecule dendrimer which has a higher molecular weight (28 kDa) and it can immobilize the hole network and results in lower solvent resistance, thus current can flow, electrons hopping occurred and hence, voltage curing can be facilitated. In this case, high zeta potential is achieved as the dispersion medium, sample in the solvent, will resist aggregation and possess stability.

When co-grafted ferrocene with PAMAM-g-diazirine get voltage-activated via zensor electrode, it has a working mechanism of the working electrode (WE), the counter electrode (CE), and the reference electrode (RE). In an electrochemical system, the working electrode is usually utilized along with the counter electrode.

The working electrode will be called either anodic or cathodic with respect to the reaction is either undergoes oxidation or reduction. Inert material such as silver and inert carbon is used for the electrode. Counter electrode only acts as an electron collector to neutralize or balance the overall charges so that current flow is possible in the circuit [35]. As for the reference electrode, it allows potential measurement of the working electrode without passing a current through.

To investigate the electron hopping is the possible either intramolecular or intermolecular distance between donor-acceptor pairs in order to perform voltage crosslinking in organic formulations, *hypothesis 3* can involve different grafting ratios of donor and acceptor pairs to study the structure-activity relationships (SARs) in cografts and blends. As such, the structure-activity relationships (SARs) studies can be determined by electrorheology test with zensor electrode setup.

2.5 Catechol groups have redox properties and are electrochemically activated well below water electrolysis

Supposition 4: *Apply the donor-acceptor theory on voltage-activated catechol grafted to dendrimer which has oxidative covalent crosslinking*

Catechol-containing adhesives are widely studied in the field of bio-adhesive as it is found as the characteristic compositions of adhesive proteins in mussels [36]. It has strong adhesion to a vast number of surfaces and some examples are metals, oxides, and polymers. The adhesion is due to the presence of catechol which eventually forms 3,4-dihydroxy-L-phenylalanine (DOPA) and dopamine [36]. Due to it has strong binding adhesion and its biocompatibility properties, it is being used as bio-adhesive. In adhesive applications, curing methods are required to crosslink the adhesive. The commonly used curing methods of catechol-containing adhesive include: (1) irreversible chemical crosslinking by using oxidizing agent (e.g. NaIO₄ [37], [38], alkaline pH [39], biocompatible catalysts [40],[41] and enzymes (horseradish peroxidase combined with hydrogen peroxide, HRP/H₂O₂, for example) [42],[43],[44]; (2) coordination crosslinking with metal ions (e.g. Cu²⁺, Fe³⁺ [45]) to form reversible network [38].

The chemical reaction between catechol and oxidizing agent starts immediately upon mixing. Although it shows adhesion crosslinking after mixing, it does not have tunable mechanical and adhesion strength when compared with UV / low voltage-activated PAMAM-g-diazirine adhesive [8, 14]. Moreover, this stimulation method is a toxic and carcinogenic risk since NaIO_4 is substantially cytotoxic to cells [46]. However, the coordination crosslinking with metal ions method forms a reversible network which is not applicable for adhesive applications as it will tend to reverse its crosslinking network with the effect of pH adjustment as well as the ion-strength. Catechol groups can oxidize into o-quinone, followed by forming covalent crosslinking such as Michael addition and Schiff base reaction [47, 48]. The established oxidative covalent crosslinking with its redox properties is a motivation to apply catechol group onto the donor-acceptor theory of voltage-activated adhesive.

The concept of applying low-voltage to electrocure diazirine-containing adhesives where the donor-acceptor pair was applied, enhancing the electron transduction when curing occurred in a non-aqueous environment. Electro curing provides the number of advantages includes: (1) do not require two parts mixing components while it is necessary for chemical curing method (2) are able to bond on opaque substrates while it is limited to on photocuring adhesive and (3) it can be used on heat-sensitive substrate while it is limited to thermal curing adhesive. However, the crosslinker in the low voltage-activated adhesive is diazirine. Hypothesized carbenes are generated upon voltage curing (-2 to -10V) for voltage crosslinking, where water electrolysis occurs at -1.6 V and H_2 , O_2 , N_2 formed as a by-product in the aqueous formulation [8][49],[50]. These H_2 , O_2 , N_2 lead to the porous structure which indirectly compromises the mechanical properties of the adhesive.

On the other hand, catechol has redox properties that exhibit electrochemically redox activation of Schiff base conditions[51],[52],[53],[54]. Redox activation can provide oxidative crosslinking of the adhesive between catechol and amines from Schiff base[55][56]. This has inspired the new donor-acceptor pairs investigation where catechol can be electrochemically oxidized at 0.8V without

the presence of water electrolysis while Schiff base accepts 2 electrons and 2 protons from the catechol dihydroxyl groups. Dihydroxy groups of catechol adhesive act as a donor when it is transformed into o-quinone while the amines from the Schiff base formation acts as an acceptor when it accepts the proton from the catechol upon voltage activation (**Figure 2.6**). Additionally, donor-acceptor concept of oxidative crosslinking can be determine using catechol-Schiff base in catechol containing adhesive as reductive crosslinking of diazirine based adhesive has demonstrated that it can undergo voltage activation. In this investigation, *hypothesis 4* can involve catechol containing adhesive to perform electrocuring with donor-acceptor concept via oxidative crosslinking and to determine whether it can obtain a balance of fast curing rate with elevated mechanical properties as no evolution of H₂, O₂, or N₂ will occur. [41].

Catechol (donor):

Schiff Base (acceptor):

Figure 2.6. Catechol acts as donor and Schiff base acts as acceptor to form intramolecular donor-acceptor pairs in catechol adhesive so that voltage activation can occur

2.6 Summation of the literature review

The important aspect of organic-based voltage-activated adhesive should reach gelation time within 5 minutes or less. The adhesive in organic formulation adapts donor-acceptor theory from the concept of metal-organic frameworks to improve its conductivity in an organic environment. Furthermore, the viscosity of the adhesive cannot be neglected too. This requires a combination of low viscosity (<1 Pa.s) before curing and high-crosslinking density after activation ($>90\%$ functional group activation) ($G'' > G'$ before curing, $G' < G''$ after curing). Therefore, the users will be able to inject or pipette the adhesive onto the target area with ease before voltage activation. On the other hand, the adhesive must be cured into solid thin film after any forms of curing methods. Therefore, oscillatory rheometry is a suitable characterization technique that can determine the transition of liquid and solid material properties. Upon voltage activation, the stiffness and elasticity of the adhesive need to be taken into consideration with the influence of ferrocene (donor) and diazirine (acceptor). In order to achieve selective nucleophiles crosslinking of the adhesive, several measures such as polar and aprotic solvent (e.g. DMSO) when voltage curing. A material strategy for a dendrimer which has many surface groups with shorter intramolecular distance so that it can be synthesized with redox crosslinker like diazirine (acceptor) and conductive additive like ferrocene (donor). Hence, it will be able to undergo voltage activation with organic solvents which are non-conductive in nature. Herein, producing an adhesive with increasing mechanical strength after activation like on-demand diazirine based adhesive or voltaglue [8],[14]. The voltage activation concept of donor-acceptor pairs does not only limit to ferrocene and diazirine. To introduce oxidative crosslinking, remove water electrolysis and N_2 gas evolution from on-demand diazirine based adhesive/voltaglue, other donor-acceptor pairs such as catechol-Schiff base is determined with structure-activity relationships studies. In terms of application, they can become one-pot voltage-activated adhesives to be used on either thermal sensitive substrates or opaque substrates.

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Chapter 3

Experimental Materials and Methodology

This chapter discusses this research project's experimental materials and methodology by presenting an experimental strategy for each hypothesis, choices of materials, and characterization techniques to determine the hypotheses and evaluate donor-acceptor voltage-activated adhesives' mechanical performance.

Experimental Strategy for Hypothesis #1 and #2

To test the hypotheses inscribed in chapter 1, this section will consist of a scope and experimental strategy. To challenge hypotheses #1 and #2, a macromolecule will be synthesized to incorporate charged functional groups that are activated electrochemically. A charge carrier, which is the independent parameter, will be grafted onto the macromolecule in different ratios to determine the effect of electron transduction. Electrorheology will determine the crosslinking kinetics of the voltage-activated adhesive by observing the dependent parameters such as storage modulus (G') and loss modulus (G''). With the presence of charge carriers (donors), crosslinking precursors (acceptors), and sacrificial oxidants (additives), an increase of storage modulus will be expected during voltage curing. A comparison of with and without charge carrier onto crosslinker-macromolecule rheology data will support hypotheses #1 and #2.

Experimental Strategy for Hypothesis #3

To challenge hypothesis #3, a macromolecule will be incorporated with charge carriers (donors), and another macromolecule will be incorporated with crosslinking precursors (acceptors) by an individual one-step synthesis reaction. The incorporated charge carriers (donors) and crosslinking precursors (acceptors) with different ratios onto the macromolecule will determine the intermolecular distance effect that controls the electron conduction during the electrorheology test. The test will be conducted to determine the crosslinking kinetics of the voltage-activated adhesive by observing the dependent parameters such as storage modulus (G') and loss modulus (G''). It will provide a shorter intermolecular distance between charge carriers and crosslinking precursors for electron transduction with higher conjugation of crosslinking precursors. Thus, an increase of storage modulus and faster gelation will be observed upon voltage curing. A comparison of different crosslinker ratios with charge carrier macromolecule rheology data will support hypothesis #3.

The macromolecule consists of the primary components: a polymer backbone, grafted charge carriers (donor), and crosslinking precursors (acceptor). A polymer backbone with molar masses over 10 kDa serves to immobilize the macromolecule, preventing diffusion at >25 wt% solid/solvent ratios. The

design of these interconnect components serves as the model system for exploring the following structure-activity relationships. Each of the components is described in detail under the materials section.

Experimental Strategy for Hypothesis #4

An experiment with donor-acceptor diazirine based adhesive in organic solvents removes water electrolysis, but the presence of nitrogen gas emission still exists. To challenge hypothesis #4, with the inspiration of donor-acceptor pairs concept for voltage activation and good redox properties of catechol, a new formulation of one-pot voltage-activated catechol-based adhesive is investigated. By grafting catechol onto a macromolecule with amino surface end groups, it creates donor and acceptor pairs. No presence of hypothesized carbene removes the nitrogen gas emission, and catechol can be electrochemically activated well below water electrolysis. Hence, the elimination of water electrolysis and nitrogen gas emission will lead to the adhesive's higher mechanical performance. By adopting the concept of donor-acceptor pairs, the test will be conducted to determine the crosslinking kinetics of the voltage-activated adhesive by observing the dependent parameters such as storage modulus (G') and loss modulus (G''). Comparing one-pot donor-acceptor voltage-activated catechol adhesive and traditional two-part curing catechol adhesive data will support hypothesis #4.

3.1 Materials

3.1.1 Choice of Polymer Backbone

A polymer backbone needs to have good solubility in solvents, multiple end groups for reaction, and high molar mass to create an immobilized network. Dendrimers are an ideal polymer as it has an open core with low viscosity, known molar mass, and exact functional group. Available dendrimers include Polyaminoamine (PAMAM), Boltorns, and hydroxyl dendrimer. Commercially available dendrimers such as G5-PAMAM is the ones that allow one-step reproducible grafting. G5-PAMAM, a synthetic dendrimer with a known structure of 128 amino surface end groups to create an immobilized network, has known molecular weight, is modifiable in high yield, and is a well-formulated adhesive (PAMAM-g- diazirine) in our laboratory.

3.1.2 Choice of Charge Carriers

Electron transduction requires positively charged carriers that need to be redox activated and non-spontaneous when exposed to the atmosphere. Several redox-active, positively charged carriers include ferrocene and cobaltocene, while neutral electron carriers include furan and thiophene. Ferrocene is chosen as a conductive positive charge carrier because ferrocene is thermodynamically stable and will only oxidize and conduct electron transduction when a low potential is applied. This is compared to which cobaltocene will oxidize spontaneously. PAMAM-g-Ferrocene (G5-Fc) also has several applications such as biosensors and biofuel cells in order to create an immobilized hole network.

3.1.3 Choice of reductive and oxidative crosslinkers

Diazirine is the preferred choice as compared with other crosslinking reagents such as aryl azides because diazirine can be reduced to diazirinyl radical/diaziridine followed by oxidation into hypothesized carbene. This uses redox balanced radicals to make crosslinking possible, smaller size, required reasonable time to voltage activate. It is stable in acidic and basic conditions with the presence of other nucleophiles such as diaziridine, nitrene, catechol, and diazonium.

Hence, G5-PAMAM acts as a polymer backbone that immobilizes the redox crosslinker and charged carrier. The source of hypothesized carbene in this project is the reduced form of diazirine (Dz), which is being utilized as a crosslinker. In contrast, ferrocene (Fc) is used to improve electron conduction. Both are co-grafted onto G5-PAMAM to serve as adhesive for fixation using voltage activation. Some of the schematic illustrations of the adhesive synthesis and its characterization techniques are presented in this section. To challenge hypothesis #1, the independent parameters are 0% and 15% of ferrocene, which is grafted onto the amino surface groups of the G5-PAMAM to promote electron transduction in a non-conductive organic solvent. As for hypothesis #2 and #3, the independent parameters are three different ratios of bromo diazirine (7%, 15%, and 22%), which is grafted onto G5-PAMAM surface groups with the

addition of sacrificial precursors to achieve accelerated crosslinking kinetic and desired mechanical properties of the adhesive by voltage activation. SEC-GPC characterization techniques will be described later in this section for molecular weight and conjugation percentages of the independent parameters (Fc, Dz). The mechanical performance evaluation methods, such as rheology tests, are designed to determine adhesive formulations' structure activity. To investigate the effect of ferrocene attachment in hypothesis #1 to #3, a comparison of ferrocene-diazirine cografts (G5-Fc-Dz), ferrocene-diazirine blends (G5-Fc + G5-Dz), and PAMAM-g-diazirine (G5-Dz) will be made. The dependent parameters such as storage and loss modulus (G' and G'') can be determined through crosslinking kinetics determined by electrorheology tests.

Catechol has redox properties which can be electrochemically activated well below water electrolysis. In adhesive research, catechol is used for bio-adhesives due to its excellent adhesion from mussels to a vast number of surfaces such as metals, oxides, and polymers. However, most adhesive research utilizes catechol with oxidizing agents such as NaIO_4 (a cytotoxic compound) for two-part chemical curing. To move on from diazirine-based adhesive with the presence of nitrogen gas evolution upon voltage activation, a new formulation with no nitrogen gas evolution of one-pot donor-acceptor voltage-activated catechol-based adhesive is investigated. 3,4-dihydroxybenzaldehyde (DBA), used as a catechol group, is grafted onto G5-PAMAM (polymer backbone) with different conjugation ratios to provide donor and acceptor pairs so that voltage activation can occur. To determine the effect of donor-acceptor pairs in hypothesis #4, a comparison of one-pot voltage-activated catechol adhesive and traditional two-part curing catechol adhesive will be made to observe its crosslinking kinetics by electrorheology test so that the dependent parameters such as storage and loss modulus (G' and G'').

3.1.4 Ferrocene-Diazirine cografts (G5-Fc-Dz) and Ferrocene-Diazirine blends (G5-Fc + G5-Dz)

Dendrimer of 5th generation Poly(amidoamine) (G5-PAMAM, $M_w=28.8$ kDa) is purchased from Dendritech, Inc, USA. 4-[3-(Trifluoromethyl)-3H-diazirin-3yl]

benzyl bromide (bromo diazirine) is purchased from TCI, Japan. Chlorocarbonyl ferrocene is purchased from PICHEMICALS, China. Dimethyl sulfoxide (DMSO) and tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) are purchased from Alfa Aesar, Singapore. Disposable Zensor® chip for electrorheometry is made of polypropylene and purchased from Zensor R&D Company, Taipei. Ferrocene cografted PAMAM-g-diazirine (G5-Fc-Dz), PAMAM-g-ferrocene (G5-Fc), and PAMAM-g-diazirine (G5-Dz) blends with different conjugation ratios are dissolved with DMSO and 0.1M Bu_4NPF_6 in 50% w/w are used as adhesives for electrorheology test.

3.1.5 Donor-acceptor based catechol grafted PAMAM (G5-DBA)

G5-PAMAM dendrimer (5th generation, 28 kDa, 128 primary amines on dendrimer) is supplied by Dendritech, USA. 3,4-dihydroxybenzaldehyde (97%, denoted as DBA) and sodium borohydride (NaBH_4) is supplied by Sigma, Singapore. Phosphate-buffered saline (10X PBS) is purchased from Gibco, Singapore and 1X PBS is prepared by diluting 10X PBS. Disposable Zensor® chips for electrorheometry are made of polypropylene and purchased from Zensor R&D Company, Taipei. PAMAM-g-catechol (G5-DBA) with different conjugation ratios are dissolved with 1X PBS in 30% w/w and used as adhesives for rheology test.

3.2 Methodology

3.2.1 Synthesis reaction of Fc cografted PAMAM-g-diazirine (G5-Fc-Dz)

Chlorocarbonyl ferrocene (Fc) is utilized as an internal conductive additive (donor) and for a synthesis reaction with the polymer substrate, G5-PAMAM. The independent parameters are 15% Fc at a fixed conjugation ratio that is grafted onto G5-PAMAM in the synthesis, followed by a different conjugation ratio of bromo diazirine (7%, 15%, and 30%) onto G5-Fc. Thus, the synthesis has a two steps reaction. The first step is to individually dissolve chlorocarbonyl ferrocene and G5-PAMAM into 5% w/v of anhydrous methanol. Then, chlorocarbonyl ferrocene solution is slowly added drop by drop into the G5-PAMAM solution. After which, trimethylamine is also added to the mixture. Then, the reaction undergoes 12 hours under a nitrogen atmosphere. Subsequently, the G5-Fc is dried

using a vacuum pump, and the dried G5-Fc crude product undergoes precipitation in a Chloroform/Hexane (1:4 in v/v) mixed solvent.

The second step of the reaction is to dissolve G5-Fc into 5% w/v of anhydrous methanol. The bromo diazirine (Dz) is also dissolved by anhydrous methanol separate from the G5-Fc. After that, the diazirine solution is added to the G5-Fc solution. Then, the reaction undergoes another 12 hours under a nitrogen atmosphere. Subsequently, the G5-Fc-Dz is dried using a vacuum pump, and the dried G5-Fc-Dz crude product undergoes precipitation in a Chloroform/Hexane (1:4 in v/v) mixed solvent. The schematic illustration of the two steps reaction is shown in **Figure 3.1**.

Step 1:

Step 2:

Figure 3.1. Schematic illustration of two-step G5-Fc-Dz synthesis reaction

3.2.2 Synthesis reaction of Fc grafted PAMAM (G5-Fc) and Dz grafted PAMAM (G5-Dz) blends

The general procedure given here for the synthesis of **G5-Fc₁₅** and **G5-Dz₁₅** dendrimers was used to synthesize G5-Fc₇, G5-Fc₂₂ and G5-Dz₇, G5-Dz₃₀.

G5-Fc₁₅ Synthesis: A methanolic solution (5 mL) of chlorocarbonyl ferrocene (0.34g, 1.33mmol) was added dropwise to a solution of G5-PAMAM (1g, 0.035mmol) in anhydrous methanol (5 mL) under a nitrogen atmosphere at room temperature. Triethylamine (0.17g, 1.77mmol) was then added, and the mixture was stirred for 16 h. The volatile materials were removed under reduced pressure, and the residue was stirred vigorously in a mixture of chloroform-Hexane (20 mL, 1:4). The resulting precipitate was filtered and dried to present G5-Fc₁₅ as an orange solid (0.6 g, 0.018 mmol). This material was used without further purification for rheological studies.

G5-Dz₁₅ Synthesis:

A methanolic solution (5 mL) of bromo diazine (Dz-Br, 0.29g, 0.66mmol) was added dropwise to a solution of G5-PAMAM (6.96g, 0.035mmol, 14.37% w/v) in anhydrous methanol (8mL) under a nitrogen atmosphere at room temperature. After stirring vigorously for 16 h at room temperature, the methanol was removed under reduced pressure. The residue was dried in a vacuum to present G5-Dz₁₅ as a yellow solid (0.6 g, 0.018 mmol). This material was used without further purification for rheological studies.

The synthesis reaction is shown in **Figure 3.2**.

Figure 3.2. Schematic illustration of one-step synthesis reaction for donor-acceptor blends, a) G5-Fc_x (donor), b) G5-Dz_y (acceptor)

3.2.3 Synthesis reaction of catechol adhesive (G5-DBA_x)

The general procedure is given here for the synthesis of **G5-DBA₂₀**

G5-DBA₂₀ Synthesis:

G5-PAMAM (0.59g, 0.026mmol, 14.37% w/v) in methanol stock solution (5 mL) is degassed with N₂ prior to any reaction to remove any dissolved O₂. DBA (0.073g, 0.053mmol) is dissolved in anhydrous, degassed methanol (5 mL) and then added dropwise to G5-PAMAM stock solution over 10 min. The reaction was stirred at room temperature under inert gas in dark conditions overnight. Conjugated products (0.5 mL) were obtained after precipitating in anhydrous diethyl ether (1.5 mL), followed by two washes of the precipitate with diethyl ether. Residual solvent is removed under a vacuum and stored at -20 °C for no longer than one week. Finally, the adhesive is wrapped with aluminum foil and kept under 4°C until utilized for other characterizations such as electrorheology studies.

The synthesis reaction is shown in **Figure 3.3**.

Figure 3.3. Schematic illustration of one-step synthesis reaction for donor-acceptor catechol adhesive (G5-DBA_x)

3.2.4 Size Exclusion Chromatography (SEC) characterization of G5-Fc-Dz cografts, G5-Fc + G5-Dz blends, and catechol adhesive (G5-DBA)

There are characterization techniques to determine the molecular weight of Fc-Dz cograft/blend adhesives and catechol-based adhesives as well as the mass conjugation of diazirine (Dz), ferrocene (Fc), and catechol units (DBA). These methods to determine the molecular weight of the samples include size exclusion chromatography - gel permeation chromatography and mass spectrometer. However, a mass spectrometer cannot characterize a dendrimer with a high molecular weight, such as 30 kDa, used in this project. On the other hand, SEC-GPC can characterize high molecular weight polymer and determine unreacted small molecules such as free Dz/Fc and free DBA by observing its elution time using RI/UV peak. Therefore, it can be used to determine whether the synthesis reaction is complete. Since Dz, Fc and DBA have UV peaks at 360nm, 440nm, and 280nm, respectively, a measurement of the mass conjugation can be calculated by beer lambert law using the UV peak areas from the UV detector. To

characterize and obtain both the mass of samples as well as the Dz, Fc, and DBA conjugation onto the dendrimer at the same time, size exclusion chromatography is preferred as it consists of a refractive index (RI) detector, an ultraviolet (UV) detector and a MALS detector (Wyatt MiniDawn, Wyatt Technology, USA). It provides comprehensive information on mass and conjugation analysis of independent parameters (Dz, Fc, and DBA), as it is equipped with three detection systems (RI, UV, and static LS).

The adhesive formulations are analysed and characterized using Agilent 1100 series high- performance liquid chromatography, SEC-GPC. A 1% w/v formic acid buffer solution is used as the mobile phase eluent at a flow rate of 1 ml min⁻¹. The SEC column (PLGel aqueous MIX-H column) is kept in a 60°C oven. Fc-Dz cograft/blend adhesive samples are dissolved in DMSO and diluted with its acid buffer eluent at a concentration of 2 mg min⁻¹, filtered by 0.2 µm microfilter, while the sample compartment is kept at ambient temperature. The G5-PAMAM's specific RI increment value (dn/dc) is 0.185. [1]. The UV extinction coefficient at 350 nm of diazirine in an aqueous medium is found to be 1009.5 mL cm⁻¹ g⁻¹ [1]. Version 5.19.1 Wyatt ASTRA software, (Wyatt Technology, Santa Barbara, CA, USA) is used to determine the experimental conjugation percentage of ferrocene and diazirine on G5-PAMAM dendrimer.

Similarly, for G5-DBA synthesis, 1% w/v formic acid buffer is used as the mobile phase eluent at a flow rate of 1 ml min⁻¹. The SEC column (PLGel aqueous MIX-H column), is kept in a 60°C oven. G5-DBA conjugated samples are dissolved in the eluent at a concentration of 2-10 mg mL⁻¹, filtered by 0.2 µm microfilter, while the sample compartment is kept at ambient temperature. The G5-PAMAM's specific RI increment value (dn/dc) is 0.185 [1]. The UV extinction coefficient at 280 nm of catechol units (DBA) in an aqueous medium is found to be 14556 mL cm⁻¹ g⁻¹ [2]. Version 5.19.1 Wyatt ASTRA software, (Wyatt Technology, Santa Barbara, CA, USA) is used to determine the experimental conjugation percentage of catechol units (DBA) on G5-PAMAM dendrimer.

The calculation of diazirine (Dz) /ferrocene (Fc) / catechol units (DBA) conjugation percentage is shown below:

The SEC-MALS characterization system is connected with a UV absorbance detector to determine the number of diazirine/ferrocene groups attached to the dendrimer.

$$C_{dendrimer} = \frac{\frac{m_d}{M_d}}{\frac{m_i \times 2^{(n+2)}}{M_c}} \times 100\% \quad \text{(Equation 3.1)}$$

$C_{dendrimer}$: Percentage of diazirine /ferrocene /catechol units' conjugation on the dendrimer. Therefore, the ratio is the total number of conjugated ferrocene and diazirine to the total number of surface groups on dendrimer polymer. There are 128 surface groups for G5-PAMAM. It is the 5th generation for this dendrimer.

n : Generation of dendrimer used for modification.

m_d : Mass of diazirine/ferrocene/catechol units calculated from the UV absorbance (350 nm/440nm/280nm) signal from SEC and UV extinction coefficient of diazirine/ferrocene.

M_d : Weight average Mw of diazirine/ferrocene/catechol units used for modification.

m_i : Total injected mass of conjugation calculated from SEC-MALS analysis.

M_c : Weight average Mw of conjugation calculated from SEC-MALS analysis.

$$A = c\epsilon l \quad \text{(Equation 3.2)}$$

A : UV absorbance at 350 nm/440 nm/280 nm

c : Concentration of diazirine/ferrocene/catechol units

ϵ : UV extinction coefficient

l : Light pass length

3.2.5 Cyclic Voltammetry (CV) of G5-Fc-Dz cografts, G5-Fc + G5-Dz blends, and catechol adhesive (G5-DBA)

Cyclic voltammetry (CV) is a method that is widely used as an electroanalytical technique in numerous chemical fields. It is often utilized to understand the redox reaction of the Fc-Dz cograf/blend adhesives and catechol-based adhesives, for

instance, the reversibility of a reaction [3]. PocketSTAT potentiostat with Ivium Technologies software is being utilized to record the cyclic voltammograms CV data for Fc-Dz cograft/blend adhesives. In contrast, AUTOLAB with Nova 2.1 software is being utilized to record the cyclic voltammogram CV data for catechol-based adhesives. There is a working electrode, glassy carbon (GC, diameter 3 mm) that undergoes a 1.0 μm diamond polishing powder, followed by a 1.5 μm , 0.3 μm and 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ polishing and a rinse using DI water after polishing. Also, a platinum counter electrode and Ag/AgCl reference electrode (filled with saturated LiCl ethanol solution for Fc-Dz cograft/blend adhesives and 3.0 M KCl solution for catechol-based adhesives). The three electrodes and the custom-built electrochemical cell are placed in a Faraday cage. For Fc-Dz cograft/blend adhesives, DMSO solution with 0.1 M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) is used as an electrolyte and all the CV data are recorded from negative potential to potential at the 100 mV s^{-1} scan rate. For catechol-based adhesives, 1X PBS solution (pH 7.2) and all the CV data are recorded from negative potential to potential at the 50 mV s^{-1} scan rate.

The concentration for bromo diazirine is 15 mM while the G5-Dz₁₅ conjugate, G5-Fc₁₅ conjugate, G5-Fc₁₅-Dz_y cograft conjugates are all 2.5 wt% in DMSO electrolyte solution. On the other hand, the DBA concentration is 10 mM while the concentrations for G5-DBA_x conjugates are 0.1 mM. All CV scans are done over three cycles to obtain steady and precise CV data. The second cycle of the CV scan is used as the finalized result.

3.2.6 Methods for real-time viscoelastic measurement

There are certain techniques to determine the real-time viscoelastic measurement and the mechanical strength of the Fc-Dz cograft/blend adhesives and catechol-based adhesives. These techniques include mechanical tensile test, lap shear tensile test, and oscillatory rheology test. Since the Fc-Dz cograft/blend adhesives and catechol-based adhesives are in viscoelastic liquid form before voltage activation and have a transition to gel-like solid after voltage activation, it is preferred to use an oscillatory rheology test to determine the mechanical strength and its crosslinking kinetics. The other techniques, such as the

mechanical tensile test, start as a solid material or a thin film. The lap shear test is unable to provide the gelation point of an adhesive during curing. On the other hand, oscillatory rheology test provides its dependent parameters such as viscosity, storage modulus (the ability of a material to store energy), loss modulus (the ability of a material to dissipate energy applied to it), the constant shear strain applied onto the sample, gelation point upon curing ($G' = G''$), real-time crosslinking kinetics of the sample, and amplitude sweep data. Last but not least, the photo/voltage activation setup for the rheometer is direct and straightforward.

3.2.7 Oscillatory Rheometry utilizing electro curing method for G5-Fc-Dz cografts and G5-Fc + G5-Dz blends

Oscillatory rheometry experiments were performed on Fc-Dz cograft/blend adhesives with 15% fixed ferrocene and different diazirine conjugation percentages (7%, 15%, and 30%) cografts and different ferrocene/diazirine conjugation percentages (7%, 15%, 22% for G5-Fc_x and 7%, 15%, 30% for G5-Dz_y), and for blends using a parallel plate rheometer setup (Anton Paar, Physica MCR 501, United States). All Fc-Dz cograft/blend adhesives are mixed with DMSO solution with 0.1 M Bu₄NPF₆ electrolyte at 50 wt %. A Disposable Zensor® chip with 3 electrodes (Zensor R&D Company, Taipei) is used as the conductive electrodes for voltage activation. After which, they are spread (20mg for G5-Fc₁₅-Dz_y cografts, 10mg each premixed for G5-Fc_x + G5-Dz_y blends before loading) on to the 3D zensor electrode which is slotted onto the zensor electrode sample holder to act as a support for rheology testing base plate to test the curing of the adhesive at a voltage of amplitude -5V. All experiments are conducted at room temperature (25°C). Experimental parameters are measured at 10% strain amplitude and 1Hz frequency by a measuring stainless-steel probe on a parallel plate 5mm in diameter (PP05, Anton PAAR, United States). It maintained a gap of 0.15-0.30mm from the surface of the zensor electrode. The portable potentiostat power supply (Vertex, Ivium Technologies, The Netherlands) is used to apply -5V in-situ within 60 minutes to the working electrodes. Precaution is required such as exposure time for DMSO solvent onto zensor chip as the zensor chip is made of polypropylene and its insulator coating is organic soluble. The experiment is conducted within 60 minutes, to avoid

dissolving the insulator coating of the zensor chip, damage, or the embedded WE to be swollen. The rheology voltage activation setup via Disposable Zensor® chip with 3 electrodes and zensor electrode sample holder is shown in **Figure 3.4**. In order to understand its structure property relationship of the G5-Fc₁₅-Dz_y cograft adhesive, storage (G') and loss modulus (G'') at -5V for G5-Fc₁₅-Dz_y cografts ($Y = 7\%$, 15% and 30% conjugation) and G5-Fc_x + G5-Dz_y blends ($X = 7\%$, 15% and 22% , $Y = 7\%$, 15% and 30% conjugation), dissolved with 50 % w/w DMSO solution with 0.1 M Bu₄NPF₆ electrolyte are measured and presented in chapter 4 and 5.

Figure 3.4. Schematic illustration of real-time rheology voltage activation setup via portable potentiostat at various voltage. The electrorheology is carried out with the aid of self-design and made accessory such as 3D printed zensor holder as shown on the right side of the figure. Designed 3D printed zensor holder is made of acrylonitrile butadiene styrene (ABS) and it fits perfectly on the rheology testing base plate. In addition, it holds the disposable zensor electrode tight for curing.

3.2.8 Oscillatory Rheometry utilizing electro curing method for catechol adhesive (G5-DBA)

Oscillatory rheometry experiments are performed on G5-DBA catechol adhesive with different conjugation percentages (10%, 20%, 30%) by utilizing a parallel

plate rheometer setup (Anton Paar, Physica MCR 501, United States). An oscillatory rheology test is the preferred method for the characterization of mechanical strength and understanding the crosslinking kinetics of the samples such as the dependent parameters (G' and G'') mentioned in **Section 3.2.5**.

All G5-DBA_x adhesive formulations (10%, 20%, 30%) are mixed with 1X PBS at 50 wt %. A Disposable Zensor® chip with 3 electrodes (Zensor R&D Company, Taipei) is used as the conductive electrodes for voltage activation. After which, they are spread (20 μ L) on to the 3D zensor electrode, which is slotted onto the zensor electrode sample holder to act as a support for rheology testing base plate in order to test its curing at a voltage of amplitude -1V. All experiments are conducted at room temperature (25°C). The experimental parameters are measured at 1% strain amplitude and 1Hz frequency with a measuring ceramic probe of parallel plate 10 mm in diameter (PP10, Anton PAAR, United States). The PP10 ceramic probe has an optimized 0.30 mm gap size, as gap sizes greater than 0.5 mm were previously found to have excessive gelation times due to diminishing electric field strength[4]. The portable potentiostat power supply (Vertex, Ivium Technologies, The Netherlands) is used to apply 0, -1, and +1 V, respectively in-situ within 60 minutes to the working electrodes to avoid the Zensor® chip's insulator coating being dissolved/damaged. The rheology voltage activation setup via disposable zensor chip with 3 electrodes and zensor electrode sample holder is shown in **Figure 3.4**. Similar parameters are employed for the chemical curing samples with 2 μ L NaIO₄ solution (dissolved in PBS in 0.1 mM) added to the 20 μ L G5-DBA_x formulations, 5 s of pipet mixing, followed by real-time rheology analysis. For self-curing samples, real-time rheology is initiated after dissolving in 1X PBS buffer. To understand its structure-property relationship of the donor-acceptor formulations, storage (G') and loss modulus (G'') at -1V for G5-DBA_x adhesive (10-30% conjugation), dissolved with 30 % w/w 1X PBS are measured and presented in chapter 6.

3.2.9 Adhesive performance assessment for donor-acceptor based adhesives (G5-Fc-Dz cografts, G5-Fc + G5-Dz blends, G5-DBA)

To optimize the adhesion strength of adhesive formulations, it is important to assess the performance of the adhesive by utilizing failure strength tests. Most materials are stressed in one direction either tensile, shear, or compressive until failure occurs on the bulk matrix or interface of the substrate (**Figure 3.5a**). Hydrogel adhesives undergo shear stress or compression stress utilized for sealants, tissue adhesives, or industrial-based adhesives, which are commonly characterized by equipment such as a rheometer, lap shear adhesion test, and tensile tester. The rheometer has a modified tensile test method which is utilized to determine the tack adhesion strength by characterized its pull-off force against the rheometer probe area.

Figure 3.5. Examples of stress direction and adhesion failure, a) tensile, compressive and shear stress direction, b) different modes of adhesion failure between substrate and adhesive which include cohesive failure, adhesive failure, substrate failure and mix failure.

A lap shear adhesion test predominately determines the resistance force to failure of the adhesion interface. The electrocuring hydrogel adhesive is sandwiched between a screen printed three carbon electrodes and a strained substrate in tensile mode while the material deforms in shear mode. A comparable methodology was utilized to evaluate adhesion strength where one adhered substrate is biocompatible materials such as PET, PLGA, collagen, bone, gelatin,

or soft tissue such as the aorta.

The two grips that hold two substrates where the adhesive is aligned and sandwiched between the fixture before performing the lap shear adhesion test are important. If the fixture alignment is not aligned, there will be an introduction of bending degree error on the adhesion joint. Degree error of peel also happens on a flexible substrate when performing lap shear adhesion test. It is also important to consider the distribution of non-uniform stress at the adhesion joint [5]. Peel tests are usually performed with flexible adhesives to measure the resistance of localized stress. Several ASTM standards of adhesive performance are brought into consideration before conducting the experiment. This includes ASTM test code F2256-05(2015) which is the standard test method for adhesion strength properties of adhesives in T-Peel by tension loading [6], ASTM test code F2255-05(2015) which is the standard test method for adhesion strength properties of adhesives in Lap Shear by tension loading [7] and ASTM test code F2458-05(2015) which is the standard test method for wound closure strength of tissue adhesives and sealants [8]. Since the donor-acceptor based adhesives are tested onto a biocompatible substrate with hard surface screen printed three carbon electrodes for voltage activation, it is appropriate to test ASTM test code F2255-05(2015) as this standard test method is suitable for non-flexible substrates.

After performing the lap shear adhesion test (ASTM test code F2255-05(2015)), there will be four types of adhesion failure which are shown in the schematic illustration in **Figure 3.5b** [9]. These types of adhesion failures are cohesive failure, adhesive failure, substrate failure, and mixed failure. Performing voltage activation on donor-acceptor based adhesives will lead to one of the adhesion failures. Cohesive failure usually occurs because of its adhesive inability to resist the adhesive's internal bonding, therefore fracturing happens through the bulk matrix and adheres on each side of the substrate. Adhesive failure occurs because there is debonding on one side of the substrates along with the adhesive. Substrate failure occurs because there is a fracture of the substrate. Lastly, mixed failure occurs when there is a combination of cohesive, substrate, and adhesive failure. To determine the adhesion failure types of the donor-acceptor based adhesives, a

lap shear adhesion test is to be conducted first, followed by a visual inspection is to observe the failure.

3.2.10 Adhesive performance against hydrated collagen films

Donor-acceptor based adhesive is cured using voltage activation. Upon electrocuring, the adhesive undergoes constant stress on surfaces. There are dry and wet surfaces. The wet surface is challenging to adhere to compared to the dry surface. If the adhesive is to use as bioadhesive to seal wounds, adhesion to wet surfaces are usually skin tissues on the human body or blood vessels/organs inside the human body. These circumstances happen with shear stresses from perspiring on the skin surface and blood flow against blood vessel walls. Therefore, it is important to assess the adhesion strength of the electrocured adhesive by utilizing a lap shear adhesion test based on ASTM F2255-05 [10]. Electro curing adhesive with the concept of donor-acceptor pairs is to be determined and established in this investigation; thus, it is suitable to test the adhesion strength on a wet surface that mimic soft tissue and perspiring skin surface. There are several substrates that are suitable to mimic wet surfaces, including hydrated collagen, gelatin and soft tissue such as the aorta while dry surfaces are bone, PET film, and PLGA film. To preliminarily assess the adhesion strength, hydrated collagen film is the choice of substrate used for lap shear adhesion experiment as it is capable of mimic soft tissue [11].

The donor-acceptor based adhesive is sandwiched between hydrated collagen film and screen printed three carbon electrodes for lap shear test setup (**Figure 3.6a**). When applying voltage activation to the adhesive, it will undergo a transition from liquid to viscoelastic material and bonded onto the substrate, in this case, is hydrated collagen film. It is then loaded onto the lap shear adhesion equipment and stress is applied in a horizontal direction at a constant strain rate till adhesion failure occurs. The adhesive strength is influenced by the material properties such as viscosity, elasticity or viscoelasticity, and the crosslinked bonds formed between the adhesive and substrate.

When the sandwiched adhesive is continuously strained and deformed, it will reach a point of the maximum load before failure. Maximum load before failure is quantified as the load at failure. Load at failure as a function of substrate area is defined as the adhesion strength. The electrocured adhesive is assessed from the shape of the curve (**Figure 3.6b**). When the adhesive remains as liquid and uncured, the material will break down weak hydrogen bonds and continue to deform at minimum load (blue line). When the adhesive is elastic, it will show load undergoes linear elastic stress and dropped to zero load shown upon failure (black line). When the adhesive is viscoelastic which is expected in our stress vs strain curve (green line), the load undergoes linear elastic stress initially; crosslinked, bonds started to break down at higher deformation where it reached the maximum load before failure, followed by an elastic behaviour observation of lower stress after the maximum load is achieved. Due to the viscous resistance of the fractured matrix, it causes an elastic behaviour of lower stress after failure. Load as a function of displacement curve can calculate the shear modulus of the electrocured adhesive. The shear stress is determined by (load/substrate area) and shear strain is determined by (displacement/thickness).

Figure 3.6. Schematic illustration of lap shear adhesion tests, a) electrocuring adhesive experimental setup, b) example of load displacement curve.

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Chapter 4*

Voltage-activated carbene based adhesives in organic solvent using the concept of donor-acceptor pairs

Chapter 4 outlines the development of a carbene based donor-acceptor adhesive system in the organic formulation. Ferrocene (donor), a charge carrier, and diazirine (acceptor), a carbene precursor, were cografted onto 5th generation polyamidoamine which dissolved into biocompatible organic solvent dimethyl sulfoxide (DMSO). The chapter summarizes the adhesive overcome voltage activation in DMSO and compared it with other organic formulations such as dimethylformamide (DMF) and PEG 400 as well as the influence in mechanical performance on adding sacrificial oxidants such as urea peroxide and benzoyl peroxide.

**This work is published in the following:*

Lu Gan, **Nigel C. S. Tan**, Ankur Harish Shah, Richard D. Webster, Sher Li Gan, Terry W. J. Steele, Voltage-Activated Adhesion through Donor–Acceptor Dendrimers. *Macromolecules* **2018**, 51 (17), 6661-6672. (**Joint author**)
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4.1. Rationale and Introduction

Hypothesis 1: *Internal polymer grafting of donor–acceptor additives is required for electron transduction and contactless electrochemistry. One of the donor-acceptor additives must be electrochemically activated into a chemical crosslinker for voltage-activated adhesion.*

Hypothesis 2: *Organic miscible peroxides will replace electrolysis oxidants hypothesized for diaziridine oxidation and subsequent carbene formation and this increases crosslinking kinetics in organic solvents.*

The adhesives global market is estimated to be worth 108B USD by 2020 and is currently dominated by two-part adhesives, consisting of liquid organic resin (*e.g.* epoxy, acrylate, or urethane monomers) and a hardener responsible for initiation and propagation of the resin polymerization[1]. The development of one-pot adhesives aims to replace two-part adhesives, as one-pot formulations have the advantages of on-demand curing, extended pot-life, automation, and simplified hardware for application. One-pot adhesives are activated (initiation of resin polymerization) through an external stimulus such as temperature or irradiation, *e.g.* snap-cure thermosets[2, 3], electron beam/gamma-cured epoxy[4], or photocured acrylates[5, 6]. Despite the advances in electropolymerization and electroconducting polymers[7-9], no structural adhesives (*e.g.* epoxy or acrylate resins) have been reported that allow one-pot electrocuring or adhesives that could be initiated by voltage with current-controlled material properties. Perhaps the most pressing challenge preventing such formulations is the electrically insulating properties of the liquid monomer and crosslinked matrix resins — reminiscent of organic solvents. Electrically conductive adhesives, utilized in the bonding of microelectronics[10-12], require conductive particle additives, including silver flakes, metal whiskers, and carbon nanotubes. Recently, our group has developed a one-pot adhesive based on carbene crosslinking mechanisms, with activation by UV photocuring[13] and low voltage (2-10V) electrocuring[14, 15]. N-heterocyclic carbenes allow the mediated polymerization of cyclic esters, epoxides, and acrylates, potentially allowing voltage-activated carbenes to induce the initiation of these thermoset

adhesives[16-18]. The electrocuring adhesive is composed of two essential moieties: (1) water/organic-soluble dendrimer, poly(amidoamine) (PAMAM),[19] which allows a high probability of intermolecular crosslinking while minimizing high viscosity linear entanglements; (2) diazirine (Dz) functional group, which generates carbene[20-23] for crosslinking either upon 320-370 nm UV irradiation[13, 24, 25] or under -1.6 V[14]. Viscoelastic properties were tunable upon Dz grafting density, dendrimer/solvent w/w ratios, or applied joules (light dose) when followed by photorheometry. The maximum reported storage modulus (G') reached ~ 100 kPa within 4 min of UV exposure.[13] Voltage was also serendipitously observed to cure this adhesive, which is defined as electrocuring.[14] Electro curing has the advantage of bonding opaque substrates where the adhesive material properties are conceivably microelectronically controlled. The PAMAM-g-diazirine dendrimer currently serves as a model system towards the development of electrocuring structural adhesives that can crosslink on-demand.

However, in terms of electrocuring PAMAM-g-diazirine formulations, the following limitations are noted: 1) limited to aqueous solutions with high salt concentrations of 0.2 to 2 M for sufficient solvent conductivity; 2) maximum storage modulus of 1 kPa vs. 100 kPa for photocuring formulations; 3) minimum electrocuring of -1.6 V in aqueous solutions simultaneously activates water electrolysis (-1.3 V) where oxygen and hydrogen evolution contributes to foaming and weakens bulk mechanical properties; 4) oxidation species generated from water electrolysis is hypothesized to activate carbene. All limitations would be curtailed or reduced if the aqueous solvent/electrolyte is replaced with a non-aqueous alternative.

To reformulate the electrocuring adhesive for organic solvents, the following three hypotheses are generated: 1) to mediate the high resistance of organic solvents, internal (polymer grafting) of donor-acceptor additives are required for electron transduction and contactless electrochemistry, the donor-acceptor theory from metal-organic frameworks (MOFs)[26] inspires this design; 2) by removing water electrolysis and its associated foaming, a higher probability of intermolecular crosslinking yields increased storage modulus; 3) organic

miscible peroxides will replace electrolysis oxidants hypothesized for diaziridine oxidation and subsequent carbene formation. Herein, we demonstrate electrocuring of an adhesive in an organic solvent for the first time. The concept of electrocuring in aqueous solution and an organic solvent is compared in **Figure 4.1a**.

Figure 4.1. Scheme illustration of (a) electrocuring in aqueous vs. organic solvents. Aqueous solutions liberate hydrogen and oxygen gases, causing excessive foaming. (b) Synthetic route of intermediate conjugates, G5- Fc_X ($X = 15\%$), and cografed conjugates, G5- Fc_X -Dz $_Y$ ($X = 15\%$, $Y = 7\%, 15\%, 30\%$).

4.2. Methods

4.2.1 Materials

G5-PAMAM dendrimer (5th generation, 30 kDa, 128 primary amines) was supplied by Dendritech, USA. 3-[4-(bromomethyl)phenyl]-3-(trifluoromethyl)-diazirine (aryl diazirine) was supplied by TCI, Tokyo. Chlorocarbonyl ferrocene was supplied by PICHEMICALS, China. Conjugates were synthesized by

following reported procedures.[13, 27] Disposable TE100 3-electrode chip procured from Zensor® R&D Company, Taipei, Taiwan.

4.2.2 Synthesis of Cografted G5-Fc₁₅-Dz_Y Conjugates

Synthesis has been previously published.[27] Briefly, chlorocarbonyl ferrocene was grafted to G5-PAMAM at a fixed grafting ratio (15%) to primary amines (denoted as G5-Fc₁₅) in anhydrous methanol, followed by aryl diazirine with grafting ratio ranging from 7%, 15% and 30% (labelled as G5-Fc₁₅-Dz_Y, Y = 7%, 15%, and 30%) with respect to surface amines. G5-Fc₁₅ and G5-Fc₁₅-Dz_Y final products were obtained by precipitating in CHCl₃/Hexane (1/4 in v/v).

4.2.3 SEC-MALS-UV Analysis

An Agilent 1100 HPLC is equipped with the in line detectors: UV/Vis, multi-angle light scattering (MALS, Wyatt Technology Corporation, US), and refractive index (RI, Agilent Technologies, Santa Clara, CA, USA) detectors. PLGel aqueous MIX-H column in a 60°C oven was applied for size exclusion chromatography with 1% w/v formic acid eluent (flow rate 1 mL/min). Dendrimer samples were dissolved in DMSO and then diluted eluent[28] to 2 mg/mL followed by 0.2 µm syringe filtration before injection (50 µL). Dn/dc of 0.185[29] was applied for PAMAM and conjugates[14]. The UV extinction coefficients are reported in **Appendix A, Table A3**, ABS spectra are reported in **Appendix A, Figure A11**.

4.2.4 NMR Spectroscopy Analysis

All desired conjugates/cografted conjugates were analysed with NMR (Bruker Advance) at 400 MHz with DMSO-d₆ utilized as the solvent. MestReNova software was applied for the peak assignment and peak integration of ¹H NMR, ¹³C NMR, ¹⁹F NMR, and 2D NMR spectra.

4.2.5 Cyclic Voltammetry (CV)

PocketSTAT potentiostat with Ivium Technologies software was employed to record the cyclic voltammograms. The custom-built electrochemical cell was

placed in a Faraday cage, equipped with a platinum counter electrode and an Ag/AgCl reference electrode (filled with saturated LiCl ethanol solution). Glassy carbon (GC, 3-mm diameter, prepared with 1.5, 0.3, and 0.05 μm Al_2O_3 polishing) served as working electrode (WE). The cyclic voltammograms data were recorded in DMSO solution with 0.1 M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) as electrolyte, at the scan rate of 100 mV/s. All cyclic voltammograms were scanned from negative potential to positive potential. The concentrations applied for aryl diazirine was 15 mM, 2.5 wt% in DMSO electrolyte solution for dendrimer samples. CV scanning was performed over three cycles and the second cycle was collected.

4.2.6 Electrorheology Instruments for Electro curing Viscoelasticity Measurements

Dynamic rheometry (Physica MCR 501 Rheometer, USA) coupled with a portable potentiostat (PocketSTAT potentiostat, Ivium Technologies, USA) was applied to activate electro curing of dendrimer samples (**Figure 4.2a**). A polypropylene-based Zensor® chip, embedded with a 3-mm diameter GC as working electrode (WE), an outer annular crescent GC as the counter electrode (CE), and an Ag/AgCl pellet as a reference electrode (RE), was selected as electrode platform. A stainless-steel rheometer probe with 5-mm diameter parallel-plate geometry “PP05” (Anton Paar, USA) was selected as a measuring probe as it covers the 3-mm diameter WE. Dissolved sample (50 wt% conjugates in DMSO solution, or 25 wt% in DMF and PEG400 solution with 0.1 M Bu_4NPF_6 electrolyte) was pipetted onto Zensor® chip. The PP05 probe (0.15 mm, 0.30 mm, and 0.60 mm gap sizes) determined the sample geometry. The loss modulus and the storage modulus of the formulations were recorded at 1 Hz strain rate for the amplitude sweep and 10% amplitude under oscillatory dynamic analysis (**SI-Video1**). The potentiostat was applied to maintain the activation voltage at -3 V or -5 V. Measurement period was controlled within 60 min to avoid the Zensor® chip’s insulator coating being dissolved/damaged or the embedded WE being swollen.

4.2.7 Electrorheology Tack Adhesion Measurements

After voltage applied, PP05 probe tensile force measurement was recorded at a velocity of 50 $\mu\text{m/s}$. The maximum load before failure is reported as the tack adhesion (N/cm^2), normalized by the working electrode area (3 mm diameter).

4.2.8 COMSOL Simulation of Electric Potential Distribution

The electric potential distribution between the work and counter electrode Zensor® electrode was generated using FEM simulation software (COMSOL V5.1). The 3D CAD model was designed in Autodesk Fusion 360 and imported as an assembly into COMSOL. The electrodes were assigned properties of glassy carbon (conductivity $2\text{E}+5 \text{ S m}^{-1}$ and relative permittivity 10)[30] and Zensor® substrate assigned nonconductive property (conductivity 0 S m^{-1}). A cylinder mimicking the conductivity of DMSO (conductivity $2\text{E}-7 \text{ S m}^{-1}$, relative permittivity 46.7)[31] was in contact with the electrode surface. -5 V was assigned to working electrodes. The electric voltage generated between the two electrodes was generated in 3D. During post-processing, the 3D data was visualized in 2D at YZ plane and a contour map was generated for the electric field. Following assumptions were made in the simulation: 1) the potential applied in the working electrode is uniform, and 2) the base on the insulator is grounded.

4.3. Results

4.3.1. Selection of DMSO, Grafted Ferrocene, and Dendrimer Allows a Conductive Medium

DMSO is selected as the organic solvent as it satisfied the following prioritized parameters: 1) an aprotic solvent that dissolves G5-PAMAM at high concentrations; 2) large electrochemical window of 5V; [32-34] and 3) acts as a hydroxyl radical scavenger, preventing interference by electrolysis of trace water[35, 36]. Other alkanols are miscible with G5-PAMAM but are not aprotic while dimethylformamide (DMF) has limited solubility.[37] DMSO allows a high concentration of 50% w/w ratio of G5-PAMAM dendrimers and all grafted derivatives, which have a similar molality as the aqueous solutions prepared previously.[14] Ferrocene (Fc) is selected as the internal additive due to its redox-

stable property and its role in electron transduction, known to promote electron transfer[38, 39] when cooperating with self-assemble monolayers[40], polymers[41-44], polymer brushes[39, 45, 46], dendrimers[47-52] and proteins[53, 54]. Dendrimers are the preferred polymer backbone, as they limit linear polymer entanglement with the intended design of low viscosity at a high concentration of 10-50 wt%. G5-PAMAM dendrimers have primary amines group constrained to the surface, which facilitates grafting and intermolecular covalent crosslinking. The cograftered G5-Fc₁₅-Dz_Y conjugates are prepared by a simple two-step synthesis as seen in **Figure 4.1b**. [27] Fc is grafted to the G5-PAMAM at a fixed grafting ratio of 15% to the G5-PAMAM amines (G5-Fc₁₅) and diazirine (Dz) is subsequently grafted on G5-Fc₁₅. Grafting the Dz on first or Fc grafting above 15% leads to solubility issues in methanol (reaction solvent) or DMSO (electrocuring solvent). The conjugation percentages of the attached dual functionalities were estimated by NMR and SEC-MALS-UV independently. G5-PAMAM and intermediate conjugates are characterized by ¹H NMR and listed in **Appendix A, Figure A1a**. Reaction efficiency and final grafting ratios are determined by ¹H NMR and displayed in **Appendix A, Figure A1b**. Size exclusion chromatography (SEC) with the appropriate detectors allows molar mass determination of grafted conjugates, and independent mass determinations of PAMAM, Fc grafting, and Dz grafting, as seen **Appendix A, Figure A2a-A2c**. The theoretical and calculated values via ¹H NMR and SEC are compared in **Appendix A, Table A1**. ¹⁹F NMR analysis of G5-Fc₁₅-Dz₃₀ probes for diazirine activation, taking advantage of the -CF₃ R-group, is shown in **Appendix A, Figure A4**. A customized electrorheology setup (**Figure 4.2a**) is applied to activate the crosslinking and record the *in-situ* real-time kinetics for both loss and storage modulus, followed by tack adhesion. Cyclic voltammetry (CV) evaluates the redox behaviour of Fc and Dz after dendrimer immobilization, to determine any shifts in voltage.

4.3.2. G5-Fc₁₅-Dz₃₀ Display Faster Crosslinking Kinetics and Increased Storage Modulus than G5-Dz₃₀ and G5-Fc₁₅ Alone

Higher activation voltages (-5 V) than aqueous electrocuring (-2 V) is applied in organic electrocuring due to the higher resistance of the organic formulations.

Electrocuring is monitored in three stages: 1) Voltage off, 0-2 min. 2) Voltage on, 2-48 min. 3) Voltage off, 48-50 min. The first stage retrieves reference viscosity and viscoelasticity of the uncured samples. The second stage records the real-time viscoelastic electrocuring properties and the third stage analyses for any continued polymerization propagation or relaxation by electric field removal. The storage moduli (G') and the loss moduli (G'') values of G5-Dz₃₀ and G5-Fc₁₅ conjugates are recorded as control (G5-PAMAM control is shown in **Appendix A, Figure A5a**) to compare with G5-Fc₁₅-Dz₃₀ conjugates, where the liquid to solid transition is shown in **Figure 4.2b (SI-Video2)**. With no voltage applied, G'' is greater than G' (liquid, viscous) with little to no drift for all samples as displayed in **Figure 4.2c** and **Appendix A, Figure A5c** (no voltage applied on G5-Fc₁₅-Dz₃₀ sample up to 40 min). Upon voltage activation, G5-Dz₃₀ displays little to no increase until 5 min of activation, with no observations of N₂ evolution. In terms of G5-Fc₁₅ conjugate, it exhibits a faster kinetics response to the applied voltage, raising about one order of magnitude in G' within 5 min. Crosslinking is speculated to be from H abstraction or free radical formation from amine-mediated electrochemical redox processes.[14, 55-58] For cograftered G5-Fc₁₅-Dz₃₀ conjugate, there are a two order-of-magnitude increase of G' and G'' values upon voltage activation with concurrent foaming (N₂ gas). G5-PAMAM controls display little to no crosslinking (**Appendix A, Figure A5a**). ¹⁹F NMR investigations exploit the -CF₃R group on the diazirine crosslinker to elucidate new covalent bonds on the carbene carbon. The ¹⁹F NMR data in **Appendix A, Figure A4** observes consumption of the aryl-diazirine-CF₃, but no new peaks are detected that would allow assignment of the crosslinking mechanism on the neighbouring -CF₃ carbene carbon. Any crosslinked samples appear to be insoluble and precipitate. This is visually confirmed in the NMR sample tubes. **Appendix A, Figure A4** displays less ¹⁹F intensity (with PF₆⁻ as internal standard) after extracting leachate from the Zensor® chip. Removing the voltage gradient results in plateaued moduli with no further modulus changes. Gas bubbles from the G5-Fc₁₅-Dz₃₀ sample accumulate on the edges of the rheometer probe as seen in **Figure 4.2b (SI-Video1)**. When removing the stainless steel rheometer probe, the crosslinked adhesive undergoes cohesive failure, as the organic gel fractures within the matrix, coating both the stainless steel probe and glassy carbon electrode as seen in **Figure 4.2b (SI-Video2)**, indicating the

interfacial adhesion is stronger on the metal and carbon/plastic surfaces than the bulk matrix crosslinking.

Figure 4.2. (a) Electrorheology schematic illustration. RE, reference electrode; WE, working electrode; CE, counter electrode; PP05, 5 mm probe, parallel-plate geometry; electric field demonstration is modelled by COMSOL software, 0.60 mm gap size is displayed as example. (b) Electro cure adhesive before and after curing. Gas evolution is visually observed. Cohesive failure causes probe/electrode fracture. (c) Real-time dynamic mechanical analysis of G5-Dz₃₀, G5-Fc₁₅, and G5-Fc₁₅-Dz₃₀ adhesives. Storage modulus (G', solid line) and loss modulus (G'', dot dashed line) throughout. Voltage off: 0-2 min. Voltage on: 2-48 min. Voltage off: 48-50 min. All conjugates formulations contain 0.1 M Bu₄NPF₆ as electrolyte. (d) Log G' comparison of G5-Dz₃₀ (grey), G5-Fc₁₅ (red), and G5-Fc₁₅-Dz₃₀ (blue) versus voltage time applied (all measurements were done in triplicate and comparison of representative data is presented in Figure 4.2c and 4.2e). (e) G' kinetics of G5-Dz₃₀ (grey), G5-Fc₁₅ (red), and G5-Fc₁₅-Dz₃₀ (blue) conjugates after 10 min voltage time applied.

4.3.3. Fc Grafting Catalyzes the Crosslinking of Electrocuring Adhesive

G5-PAMAM with Fc internal additives attempt to mediate the heterogeneous electron transfer reactions between the electrode and aryl diazirine grafted dendrimers, as well as enabling homogeneous electron transfer reactions between

linked and non-linked grafted dendrimers. The storage modulus for G5-Dz₃₀, G5-Fc₁₅, and G5-Fc₁₅-Dz₃₀ conjugates are compared at 0, 1, 5, and 46 min in **Figure 4.2d**. At 0 min, all conjugates display similar viscoelastic liquid properties, where $G'' > G'$, and G' is ~ 1 -2 Pa. Without the internal conducting additive of Fc, G5-Dz₃₀ conjugate (grey columns) exhibit a sluggish increase of G' that is limited to 55 Pa (standard deviation, $\delta = \pm 10$ Pa) at 46 min. Similar moduli are observed with free, non-grafted Fc dissolved within G5-PAMAM/DMSO (data not shown). Comparing with G5-Dz₃₀ conjugate, G5-Fc₁₅ conjugate (red columns) exhibits a faster G' increase within 1 min but halts at a maximum 105 Pa ($\delta = \pm 25$ Pa) at 46 min. G5-Fc₁₅-Dz₃₀ conjugate (blue columns) has an exponential rise in kinetics within the first few minutes but linearly increases thereafter to 3400 Pa ($\delta = \pm 500$ Pa) at 46 min. To compare the reaction rates as a function of time, G' kinetics are obtained through the differentiation of the electrorheometry plots from **Figure 4.2c** and listed in **Figure 4.2e**. Both Fc grafted conjugates exhibit sharp peaks within 5 min voltage activation, indicating the activation of redox processes that lead to electric field alignment or crosslinking of conjugates, while the G5-Dz₃₀ requires a delay before any increase in storage modulus is observed. This observation indicates that with grafting of Fc, redox reactions activate immediately upon voltage through the established electric field.

4.3.4. Leachate Evaluation Displays a Synergistic Mass Loss of G5-Fc₁₅-Dz₃₀ Matrix

The G5-PAMAM conjugates are evaluated before and after electrocuring to compare the soluble fractions (leachate) as a means to estimate insoluble intermolecular crosslinking, as seen in **Figure 4.3a-4.3b**. If little to no crosslinking occurs, then the major mass fraction will dissolve into neat DMSO after the potential is applied. If intermolecular covalent bonds are formed, the crosslinked matrix will be insoluble or filtered out (0.2 μ m) in the SEC sample preparation. After electrocuring, G5-PAMAM, G5-Dz₃₀, and G5-Fc₁₅ conjugates have greater than 70% mass retention as measured by the RI detector (**Figure 4.3a**). Quantitation of the grafted Fc and Dz by UV spectroscopy corroborates the retention of the conjugate's mass (**Figure 4.3b**). With no grafted

Fc, G5-Dz₃₀ displays an 18% reduction of Dz, corroborating the rheometry results presented above and in **Figure 4.2c**. This is likely due to the diminished absorption coefficient from diazirine ($\epsilon_{353} = 266$) to reduced diaziridine ($\epsilon_{353} = 0$).^[59] G5-Fc₁₅-Dz_Y conjugates display strong inverse correlations between percent Dz grafted and percent retained after electrocuring for dendrimer mass (Pearson's $r = -0.96$) and Dz mass (Pearson's $r = -0.99$), signifying that the higher the percent Dz grafted, the less that is retained after electrocuring. Less than 35% of conjugates mass is recovered after electrocuring and less than 50% of the grafted Dz is retained. The discrepancy between the two measurements likely results from residual UV absorbance of Fc grafting that remains after carbene crosslinking. Regardless, the ratio between uncured and cured UV absorbance (ABS) grows with increasing Dz grafting percentage.

Figure 4.3. (a) Refractive index (RI) analysis (area under curve) before (white) and after (grey) 46 min electrocuring (all measurements were done in triplicate). Refractive index is proportional to dendrimer mass. (b) ABS analysis (area under curve @ 360 nm) before (white) and after (grey) 46 min electrocuring (all

measurements were done in triplicate). Absorbance is proportional to diazirine mass. (c) Comparison of G5-Fc₁₅-Dz₃₀ at a range of sample thickness. (d) Comparison of G5-Fc₁₅-Dz_Y gelation times from 2-8 min voltage applied. Storage modulus (G', solid line) and loss modulus (G'', dot dashed line) throughout. Voltage off: 0-2 min. Voltage on: 2-48 min. Voltage off: 48-50 min. Gelation of G5-Fc₁₅-Dz₇: 6.3 min. Gelation of G5-Fc₁₅-Dz₁₅: 4.4 min. Gelation of G5-Fc₁₅-Dz₃₀: 3.1 min. All conjugates formulations contain 0.1 M Bu₄NPF₆ as electrolyte.

4.3.5. Electro curing Kinetics and Mechanical Properties are Dependent on Sample Thickness

The gap size between the rheometer probe and Zensor® chip varies the sample thickness during the electro curing measurements of G5-Fc₁₅-Dz₃₀ conjugates. Thus, the electric field between the working electrode and the counter electrode is varied. The demonstration of an electric field between probe and working electrode is displayed in **Figure 4.2a**. Applying Gauss's law ($\Phi_E = Q/\epsilon_0$, where Φ_E is the electric flux through a closed surface enclosing any volume, Q is the total charge enclosed within the volume, ϵ_0 is the electric constant), the current density or charge flux will exponentially decrease as the sample thickness increases. If this relationship holds true in electro curing kinetics, inhibition of adhesive crosslinking occurs furthest away from the electrodes — at the rheometer probe interface given the planar geometry of the two surface electrodes on Zensor® 3-electrode chip. Three sample thicknesses of 0.15, 0.30, and 0.60 mm are evaluated for G' and G'' with the fastest curing sample of G5-Fc₁₅-Dz₃₀ held at -5 V (**Figure 4.3c**). A strong linear correlation (Pearson's $r = 0.98$) exists between gelation time and sample thickness, and a linear inverse correlation (Pearson's $r = -0.93$) exists between gelation time and final G' values (**Appendix A, Figure A6b**). The fastest electro curing kinetics is observed at 0.15 mm, which also has the highest G'. Sample thickness introduced an increasing curing latency across all samples, which is also observed in **Figure 4.3d** when Dz grafting is varied.

4.3.6. Crosslinking Kinetics of G5-Fc₁₅-Dz_y is Dependent on Grafting Percentage

The gelation time defined where $G'' = G'$, is a simple measure of comparing crosslinking kinetics, where reduced time points indicate a faster transition from viscoelastic liquid to solid behaviour. Gelation times occurred at 6.3 min, 4.4 min, and 3.1 min, for G5-Fc₁₅-Dz₇, -Dz₁₅, and -Dz₃₀, conjugates respectively, as analysed in **Figure 4.3d**. The gelation time decreases with the increasing amount of grafted Dz on G5-PAMAM and the correlation falls into a strong inverse relationship (Pearson's $r = -0.96$, **Appendix A, Figure A6a**). The strong inverse correlation of percent Dz vs. gelation time is consistent with the aqueous observations of -0.97 reported previously.[14]

4.3.7. Fc Cografted Conjugates Achieves Higher Storage Modulus in Organic Solvents than Aqueous Solvents

Electrocuring kinetics of several representative adhesive samples are compared in **Figure 4.4a**. G5-Fc₁₅ and G5-Fc₁₅-Dz_y conjugates are aqueous insoluble therefore not available for aqueous electrocuring comparisons (Fc-containing conjugates are only soluble at low concentration, 2 mg/mL, acidic mediums). G5-Dz₃₀ conjugates achieve ~1000 Pa storage modulus when electrocured in aqueous solvents (1X PBS buffer as electrolyte). For comparison, G5-Dz₁₅ data is replotted from the previous report[14] with the concentration of 25 wt% in 1X PBS. The G' values reach over 80% of their maximum values after only 1-min voltage activation and achieve G' values of 1000 ± 80 Pa. The G5-Dz₃₀ conjugate exhibits a more inert response when electrocured in DMSO. Compared to G5-Dz₃₀, the G5-Fc₁₅-Dz₃₀ conjugate increases at a slower rate with an achieved G' value of 300 ± 50 Pa after 5-min voltage applied. The advantages of non-aqueous electrocuring allow extended curing times without the electrolysis reactions involved in an aqueous solvent. With the extended voltage stimulus, a final G' value of 3400 ± 500 Pa after 46-min is attained. Furthermore, Fc cografted conjugates are found electrocuring in DMF and polyethyleneglycol-400 (PEG400) (which serve as organic solvents with low dielectric constants: 37 and 16[60], respectively) as well (**Figure 4.4a** and **Appendix A, Figure A5b**): final G' values achieve 500 ± 50 Pa in DMF and 200 ± 30 Pa in PEG400 with 25 wt%

concentration of G5-Fc₁₅-Dz₁₅ conjugates. And both solvents display viscous (liquid) properties before curing, with electrocuring gelation times similar to DMSO.

4.3.8. Peroxide Additives are Not Required for Eletrocuring

Moreover, to challenge the hypothesis that additional oxidants are required for carbene formation,[22] two organic peroxide additives, either urea peroxide (UPO) or benzoyl peroxide (BPO) are added to the formulation. This serves to replace the anodic oxidants created by water electrolysis, which is theorized to aid in carbene formation of the aqueous formulations. The electrocuring kinetics is compared by monitoring the G' values at three-time points upon voltage application (**Appendix A, Figure A7**): 0 (initial), 1, 5, and 46 min. The addition of UPO or BPO into G5-Dz₃₀ and G5-Fc₁₅-D_Y samples leads to no improvement in kinetic rates or modulus values when compared with no-peroxide control (**Appendix A, Figure A7a**). Increasing molar ratios of UPO to G5-Fc₁₅-Dz₃₀ are observed to hinder the 1 min kinetics, but no consistent correlations are noted after 5 or 46 min (**Appendix A, Figure A7b**).

Figure 4.4. (a) G' values of electrocuring formulations: G5-Dz₃₀ and G5-Dz₁₅ conjugates in aqueous solution (1X PBS, at -3 V and -2 V voltage activation, respectively) vs. G5-Dz₃₀, G5-Fc₁₅, and G5-Fc₁₅-Dz₃₀ conjugates in DMSO and G5-Fc₁₅-Dz₁₅ conjugates in DMF and PEG400, at -5 V voltage activation. *G5-Dz₁₅ conjugate is replotted from reference, the concentration of G5-Dz₁₅ conjugates is 25 wt% in PBS. G5-Fc₁₅ and G5-Fc₁₅-Dz_Y conjugates are insoluble in PBS, pH 7.4. (b) Tack adhesion of electrocured formulations (grey columns) and uncured control (white columns). Significant differences are indicated by *p < 0.05, no significant difference is indicated by NS. All measurements were done in triplicate.

4.3.9. Tack Adhesion Strength Correlates with Diazirine Grafting

Tack adhesion evaluates the electrocured formulations through tensile normal forces (F_N). **Figure 4.4b** displays the maximum tack adhesion strength (normal stress at failure) of all adhesive formulations, comparing with control samples: G5-PAMAM, G5-Dz₃₀, and G5-Fc₁₅, respectively. The control samples display 2.5 N/cm², which is the same as the formulations without applying any voltage as seen in **Figure 4.4b**. Cografted G5-Fc₁₅-Dz_Y conjugates exhibit an obvious increase of tack adhesion strength, especially for G5-Fc₁₅-Dz₃₀. A strong linear correlation of tack adhesion strength and Dz conjugate with a linear fit of Pearson's $r = 0.985$ is displayed, indicating that the adhesion strength is tunable as a function of Dz grafting.

4.3.10. G5-Fc₁₅-Dz_Y Has Irreversible Dz Reduction with Reversible Fc Oxidation

Quasi-reversible behaviour is observed for aryl diazirine in DMSO, but in the presence of 1.0% acetic acid (acting as the proton donor)[14, 20], the aryl diazirine is chemically irreversibly reduced to aryl diaziridine in **Figure 4.5a**. The cyclic voltammograms of G5-Dz₁₅ conjugate retain the properties of free aryl diazirine: in the absence of the acetic acid, G5-Dz₁₅ conjugate has quasi-reversible redox behaviour; with the addition of acetic acid, only the reduction peak for the conjugate is observed in **Figure 4.5b**. Redox potentials of all samples are listed in **Appendix A, Table A2**, vs. Ag/AgCl (saturated LiCl in ethanol). Free Dz (aryl diazirine) compared to dendrimer grafted Dz (G5-Dz₁₅) in the absence of acid shows a positive shift in reduction potential (E_{pc} , from -1.6 to -1.25) and displays a negative shift in its oxidation potential (E_{pa} , from -1.0 to -1.1) when the scan direction is reversed so that the reduced form is oxidized back to the starting material. Overall, this results in a decreased value of peak separation ($\Delta E_p = E_{pa} - E_{pc}$) of 0.45 V. ΔE_p values between free Dz and grafted Dz, indicate less electromotive force is required to create the diazirinyl radical, but with more reductive potential for grafted Dz. Free Fc and G5-Fc₁₅ in **Appendix A, Figure A9b-A9c** displays an opposite trend—more electromotive force is required to oxidize grafted Fc, ultimately creating ferrocenium (Fc⁺) that has a higher oxidation potential with ΔE_p values ranging from 0.28 to 0.16.

G5-FC₁₅-Dz_Y cyclic voltammograms are tested without the presence of acetic acid in **Figure 4.5c**. Peaks are observed for Fc in the range of 0.20 V to 0.80 V and Dz in the range of -1.20 V to -1.60 V. Peak intensity of Dz rises with increasing Dz grafting. No oxidation peaks for Dz grafting is observed, indicating the redox behaviour became chemically irreversible with the grafting of Fc. Fc is not a proton donor but is a source of electrons, and Fc⁺ serves as a hole charge carrier and potential oxidant. The ΔE_p of Fc in G5-FC₁₅-Dz₃₀ is 0.17 V wider than the rest of cografed conjugates, which corresponds to the doubling of Dz grafting.

The quasi-reversible electrochemical reaction is exhibited in **Appendix A, Figure A10**, and the proposed voltage-activated crosslinking reactions are depicted in **Figure 4.5d-4.5e**. There are three possibilities for carbene to form the crosslinking: (1) insert to amide groups and (2) insert to amine groups of G5-PAMAM, or (3) self-crosslink to form azine. For conjugate samples, the current signals decay after three cycles as seen in **Appendix A, Figure A9**. Possible explanations include electrode grafting or amine oxidation.[55-58, 61-63]

Figure 4.5. Electrochemical behaviour of: (a) Aryl diazirine and (b) G5-Dz₁₅ conjugates in the absence (solid line) and presence of 1.0% acetic acid proton donor (dot dashed line). (c) G5-FC₁₅ and G5-FC₁₅-Dz_Y conjugates. All cyclic voltammograms were recorded in DMSO containing 0.1 M Bu₄NPF₆ as electrolyte at glassy carbon

electrode ($d = 3$ mm). Scan rate: 100 mV/s. (d) Possible crosslinking reactions between diazirine ($\rightarrow$ carbene) and G5-PAMAM dendrimers. (e) Simplified scheme for depicting the proposed hole conduction and carbene activation.

4.4. Discussion

4.4.1. Fc and Dz Dendrimer Cografts Enable Electro curing in DMSO

For the first time, a model system is demonstrated towards the development of voltage-activated crosslinking adhesives in a water-free environment. Structure-activity relationships of fifth-generation polyamidoamine (G5-PAMAM, 30 kDa) grafted with diazirine (Dz, a carbene precursor) and ferrocene (Fc, an internal conductive additive) were applied towards an electro curing adhesive in DMSO organic solvent. The chemically irreversible reduction was only possible with Fc and Dz co-grafting, with an observed synergistic rise in storage modulus compared to either grafted carbene precursor or internal additive alone. G5-Fc₁₅-Dz_Y (Y=7%, 15%, and 30%) conjugates exhibited liquid properties and crosslinked to a viscoelastic solid within minutes of low voltage excitation, which are hallmarks of an ideal one-pot event activated adhesive. Strong correlations were observed with respect to Dz grafting ratio on cured modulus and gelation time, which provides a pathway for tunable mechanical and adhesive properties. Variations in the electric field and gap thickness (**Figure 4.2a** and **Figure 4.3c**) displayed similar correlations on electro cured modulus and a kinetic latency time. Additional oxidants were not required and did not serve to increase storage modulus kinetics or cured storage modulus. Fc grafting induces irreversible grafted Dz reduction and Dz shifts the redox properties of grafted Fc.

The previous investigation of aqueous electro curing of PAMAM-g-diazirine,[14] attempted to replicate the voltage-activated crosslinking in organic solvents (*e.g.* acetonitrile) with no success, but with the caveat that the diazirine ($-N=N-$) had been reduced to the diaziridine (HN-NH), while subsequent oxidation of diaziridine to carbene failed. This may be attributed to the lack of catalyst to activate the diaziridine into carbene. When G5-Dz₃₀ was electroactivated in DMSO, a measurable increase in G' was detected, but SEC indicates little to no crosslinking — dendrimer mass and diazirine concentration remained approximately the same (**Figure 4.3a-4.3b**). A similar result was observed in ^{19}F

NMR examinations for before and after G5-Dz₃₀ electrocuring: the intensity of -CF₃ from Dz units change less than 8% (**Appendix A, Figure A4c-A4d**). The G' increase may result from attractive hydrogen bonding or electrostatic attraction of diazirinyl/amine. G5-Fc₁₅ conjugates displayed a rapid G' response upon the voltage application that was similarly found to have little to no crosslinking by SEC. Electrooxidation to PAMAM-g-ferrocenium may lead to electrostatic or redox-mediated aggregations (*e.g.* Fc⁺-electrolyte bridges), forming reversible non-covalent bonds as seen in other Fc grafted polymers.[64, 65]

4.4.2. Enhanced Charge Transport of Redox Dendrimer and Hole Network Activated Carbene Crosslinking

Due to the kDa molar masses of G5-PAMAM conjugates ($M_w > 30$ kDa) its improbable for PAMAM dendrimers to diffuse from the cathode to the anode (~0.1 mm apart) at these time scales. We postulated that the carbene crosslinking observed in the DMSO is attributed to two supporting mechanisms: the enhanced charge transport of redox dendrimers and the immobilized donor-acceptor network created through Fc/Fc⁺ oxidation and Dz/Dz⁻ reduction[66, 67]. The rapidly created donor-acceptor network allows the electron transduction across the bulk matrix. With the Fc cogenerated on the conjugates, Dz is irreversibly reduced to diaziridine without the proton donors, suggesting that Fc/Fc⁺ acted as the hole transfer carrier for electron hopping and its redox property allowed it to be cyclically regenerated. Our system exploited Fc's unique behaviour when grafted to dendrimers. Fc-grafted onto self-assembled monolayers results in multiple redox peaks attributed to varying electrolyte micro-environments.[68] However, when Fc is grafted on dendrimers, the multiple redox centers appear as a single but broad redox peak (**Figure 4.5c**).[67] Once the voltage was activated, Fc-Dz units were converted to Fc⁺-Dz⁻, preparing donor-acceptor charge transfer zones and rapidly creating a conductive network within minutes (**Figure 4.5e**). We speculated that the rise in resistance over time was due to the charge imbalance caused by the conversion of negatively charged diazirinyl radical to carbene, which is formed on electron reduction of Dz ($\text{Fc} + \text{Dz} \rightarrow \text{Fc}^+ + \text{Dz}^- \rightarrow \text{Fc}^+ + \text{carbene}$). Diazirinyl is a strong reductant with a relatively long

lifetime ($t_{1/2} = 46$ s @ 10°C) which can donate an electron to Fc^+ or other adjacent diazirines.[21] The electron conduction between cathode and anode was made possible by dendrimer grafted Fc (but not free Fc). The addition of the oxidants urea peroxide and benzoyl peroxide to G5-Dz or G5- Fc_{15} -Dz_Y had no discernible effect on storage modulus, suggesting that diaziridine may not be the carbene forming intermediate. This implies that the rate-limiting reaction is then the reductive diazirinyl radical to carbene via an unknown mechanism. Indirect observations support the statements above: higher grafting Dz ratios decrease Dz^- - Fc^+ distances, increasing instantaneous electron transduction with a corresponding decrease in gelation time (**Figure 4.3d**). As the Dz^- are converted to functional groups (*e.g.* carbene, azine) with reduced charge transfer, resistance rises, and current drops over time (**Appendix A, Figure A8**). Diminishing electric field (**Figure 4.2a**) will have a direct effect on the kinetics of electron hopping (*vs.* spontaneous propagation), which was observed with increasing sample thickness between the working electrode and rheometer probe (**Figure 4.3c**). The mechanism of intermolecular dendrimer crosslinking is likely to be a complex mixture of carbene (C-H or N-H) insertion and azine crosslinking.[69, 70] The mechanism of crosslinking allowed adhesion on both stainless steel (rheometer probe) and polypropylene/glass carbon substrate (Zensor® chip electrode) — cohesive failure coated a layer of adhesive on both substrates when separated (**Figure 4.2b**). Metallocenes other than ferrocene may improve the hole network properties in organic electrocuring. Cobaltocenium for example has a much lower oxidation voltage (-1.3V compared to Fc/Fc^+), but is relatively unstable in air and moisture environments.[71, 72] While cobaltocenium as an 18-electron cationic metallocene is a desirable candidate for hole carrier.[73-75] Other redox centers, such as nitroxyl, phenoxyl, carbazol, quinones, viologens, and hydrazyl, are also possible to balance the donor-acceptor charge transfer to modify Dz grafted adhesives[42]. Future work will address suitable redox mediators applicable to redox-active gels to further improve the electron transduction efficiency and lower the time-dependent resistance[76].

The simple two-step synthetic strategy was designed to prevent Fc and Dz cograftering on one terminal amine. Investigations by 2D NMR analysis (**Appendix A, Figure A3**) found no evidence of disubstituted amines with Fc

and Dz, although the previous investigation of high ratios (20-30%) of diazirine grafting yielded disubstituted amines, which cannot be ruled out herein.[13] The theoretical spacing between Fc and Dz sites is less than 2 nm at the 22-45% grafting (based on $n=120$ polyhedron, 5.4 nm diameter). The spherical nature of PAMAM dendrimers allowed the adhesive formulation to achieve a high concentration while maintaining viscous flowable properties ($G'' > G'$). With 50 wt% concentration (maximum concentration in DMSO), dendrimer conjugates are considered 'random closely packed', which is favourable to intermolecular covalent bonding.[77] Both intermolecular and intramolecular electron transfer was aided between redox-active sites and working electrodes by electron tunneling, which is facilitated by dendrimer networks.[14, 78-80]

4.4.3. G5-Fc₁₅-Dz_Y Conjugates Motivated by Donor-Acceptor Pairs in Metal-Organic Frameworks (MOFs)

The electrocuring adhesive formulations herein have donor-acceptor concentrations over $10E+20 \text{ cm}^{-3}$ which meets the high charge density requirement of metal-organic frameworks, (MOFs, $>10E+15 \text{ cm}^{-3}$)[81]. The conductive mechanisms of MOFs rely on long-range charge transport pathways through electron hopping or band transport. The choice of internal additives attempts to mimic the donor-acceptor mechanisms known in MOFs, where ferrocene serves as the metal ion and diazirine as the organic linker, albeit in a non-coordinated, dynamic liquid. Similar to MOFs, the design of metal ions and organic linkers will influence the conductive properties, which is part of our future work. The energy diagram (**Appendix A, Figure A12**) for Fc^+/Dz^- diluted in DMSO/electrolyte displays a $2.0 \pm 0.1 \text{ eV}$ bandgap. It is unknown if the charge transport is predominantly intermolecular or intramolecular, hence further exploration of cograftering vs. mixing is required. Many donors (*e.g.* naphthalene, perylene, and tetrathiafulvalene) and acceptors (*e.g.* tetracyanoethylene, fullerene, quinone, and pyromellitic dianhydride)[82] are known and will require optimization of donor-acceptor ratios to yield high crosslinking moduli and complete Dz/crosslinker consumption.

4.4.4. Benefits of Organic vs. Aqueous Electro curing

The selection of DMSO as solvent serves as a model system towards transitioning from aqueous to organic environment. Organic formulations allow higher activation voltage (-5 V) than aqueous (i.e., -2 V), with the additional benefits of a higher concentration, viscous properties, and higher storage modulus upon electro curing. Aqueous electro curing requires the lowest voltage possible due to competing electrolysis. One of the most important differences between organic and aqueous electro curing was the decrease in gas generation and subsequent foaming by H₂ and O₂, while DMSO solvent has only N₂ as a gaseous by-product (**Figure 4.1a**). The gelation time points of DMSO were comparable to aqueous (both 3-6 min) but the final G' values are 4-5 times higher, due to longer durations of voltage activation made possible by preventing water electrolysis. However, the G' values are still a fraction of what is achievable by photocured PAMAM-g-diazirines.[13, 83] This is in part due to the plasticization by DMSO and incomplete crosslinking due to the decreasing current migration over time (**Appendix A, Figure A8**). Storage modulus may be improved through a number of techniques. For example, the choice of oligomer/ionic solvents, incorporating oxidative crosslinking and optimized electrolyte mediators, which will be the focus of our future work. DMSO is a common plasticizer for bioerodible polymers (*e.g.* poly(lactic-co-glycolic acid), PLGA, with MPa shear modulus) that become viscous liquids when dissolved at 50-60% DMSO.[84-86] DMSO is commonly employed for in situ drug depots because of its many advantages: one of the lowest levels of tissue toxicity among suitable depot solvents, reduces burst release, completely miscible with water, and dissolves a range of drugs (lysozyme protein, cisplatin inorganic, hydrophobic risperidone) and bioerodible polyesters.[86-90] One of the drawbacks of in situ implants is their lack of reproducible depot shape, which could be directly alleviated with the electro curing principles presented herein.[88] PEG400's low toxicity is exploited for a wide range of purposes including cosmetics, cryogenics, drug depots, and solvent extraction.[91-93] Besides DMF and PEG400, other aprotic solvents such as dimethyl carbonate, propylene carbonate, 1-methyl-2-pyrrolidone, are also possible for a combination of drug depot and organic electro curing due to their biocompatible properties.[94-97]

4.4.5. Immobilization of Biosensors and Nanobatteries Based on Dendrimer-Fc Conjugates

Fc and Dz were previously linked together as a small molecule conjugate to functionalize graphene and carbon nanotube surfaces.[98-100] Dz was exploited for its photocuring carbene crosslinking to label Fc on the carbon allotrope, whose redox behaviour served to characterize the surface modification on the conductive electrodes. Dendrimer Fc conjugates structures have been applied towards anion and cation sensing, detection of specific oligonucleotides, molecular nanobatteries, and surface redox investigations.[48-51, 67, 71, 101-104] The stimuli-sensitive feature of Fc combined with the tailorable and easily-grafted dendrimer architecture allows electrochemical approaches not possible with other designs. Our results demonstrated how one-step grafting of aryl-diazirine to dendrimer-Fc conjugates may allow voltage-activated immobilization, expanding the dendrimer-metallocene application window on to sensitive substrates and into new environments where thermo- and photo-curing methods have prevented application. Moreover, DMSO's miscibility with both aqueous and organic solvents allows ease of extraction after matrix crosslinking enabling the possibility of both flexible electronics, implantable biosensors, and current activated drug depots, which will be explored in our future work.

4.5. Conclusion

A series of voltage-activated adhesive dendrimers with donor-acceptor internal additives was developed towards a model system for one-pot adhesives. DMSO, DMF, and PEG400 were found to act as a suitable solvent for electrocuring that avoided electrolysis gaseous by-products, allowing extended times (up to 45 min) of voltage curing not possible in aqueous solutions. Electrorheology measurements employing relatively high voltages of -5 V activated conductive $\text{Fc}^+ \text{-Dz}^-$ organogel networks in insulating solvents. The conductive milliamperic current-carrying network activates carbene insertion crosslinking. The one-pot electrocuring adhesive allows application on opaque and thermally sensitive substrates under ambient conditions, opening new applications in flexible electronics, implantable biosensors, and adhesive drug depots.

4.6. Associated Contents in Appendix, Supporting Information

Quantitation of Fc and Dz dendrimer grafting, including ^1H NMR spectrum and SEC-MALS-UV spectrum; 2D NMR of G5-Fc₁₅-Dz₃₀ analysis; ^{19}F NMR analysis of before and after electrocuring adhesive formulations; Electrorheology results of G5-PAMAM and G5-Fc₁₅-Dz₃₀ as control, and G5-Fc₁₅-Dz₁₅ electrocuring in DMF and PEG400 solvents; Linear correlations in G5-Fc₁₅-Dz_Y crosslinking kinetics studies; Adhesive formulations electrocuring in the presence of peroxide additives; Current and resistance of formulations measured in electrorheology; CV scanning for G5-Fc₁₅ conjugates; Quasi-reversible electron transfer path in CV scanning; UV absorbance spectra of conjugates with a summary of the UV coefficient values; Energy diagram of Fc and Dz in conjugates; Videos of electrorheology testing, are supplied as Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Chapter 5*

Structure-Activity relationships of cograft vs blends Fc and Dz dendrimer-based adhesives in the organic solvent

Chapter 5 outlines the blending strategy expands the efficiency of evaluating the donor-acceptor system in voltage-activated adhesives when performing the structure-activity relationship (SAR) studies. Cograft formulation strategy reduces throughput efficiency, organic solubility, and requires additional purification of the grafted dendrimers. The blending strategy is proposed for setting-up of the donor-acceptor conductive network by exploiting a flexible blending design, providing faster throughput of structure-activity analyses with less synthetic investment. The blend method investigates the ampere dependent storage modulus and gelation time as a function of both donor and acceptor concentration.

**This work is published in the following:*

Nigel C. S. Tan, Animesh Ghosh, Terry W. J. Steele, Structure–Activity Relationships of Voltaglu Organic Blends. *Macromol. Rapid Commun.* **2020**, 2000188. **(First author)** Reproduced with permission, Copyright (2020) John Wiley and Sons.

5.1. Rationale and Introduction

Hypothesis 3: *Correlation of Dz grafting percentage to adhesion strength is attributed to the number of carbenes, which are hypothesized to be the crosslinking moiety. Increasing Fc grafting is hypothesized to increase the electron mobility via lowering the e- tunnel length.*

Voltaglue, a diazirine grafted PAMAM dendrimer (G5-Dz) serves as a model system in the field of carbene-based bioadhesive research to investigate and develop adhesives that cure under low voltage.[1] Crosslinking in Voltaglue can be initiated with diverse electrode geometries such as disposable 3-electrode chips,[1][2] bioresorbable interdigitated 3D-graphene electrode (IDE)[3] and semiconductive substrates.[4] Despite these advances, Voltaglue has some limitations (when stimulated/electrocured) in aqueous environments: (1) it displays low storage modulus (1 kPa) in comparison to photo- and thermal-curing (100 kPa) and (2) the water electrolysis that takes place at -1.3V may lead to excessive foaming. Recently we reported that most of these disadvantages could be overcome by employing donor-acceptor based electrocuring in an organic environment.[2] The donor-acceptor pairing, inspired from metal-organic frameworks (MOF), creates a conductive network enabling electrocuring in insulating solvents.[5] The pairing of Fc/Dz (ferrocene/diazirine) can function as an auto redox donor-acceptor pair when subjected to voltage in biocompatible organic solvents such as DMSO, DMF, and PEG400. The Fc and Dz used as donor and acceptor respectively are cogenerated onto G5-PAMAM to prevent migration to the electrodes.[2] When subjected to a voltage, diazirine undergoes 1 e reduction to diazirinyl radical while ferrocene donates 1 e to create a hole-mobile conductive network across the adhesive matrix. The speculated singlet carbene generated from the transient diazirinyl radical results in covalent bond formation on a variety of organic functional groups e.g. C-H, O-H, N-H, C=C, etc.[6] However, preparation of the cogenerated dendrimer involves laborious two-step synthesis and results in reduced solubility compared to the single grafted dendrimer. Instead of cogenerated, a blending strategy would expand the efficiency of evaluating donor-acceptor systems when performing structure-activity relationship studies.

Herein, the blending of donor/acceptor grafted dendrimers is compared to cograftering formulations to evaluate if electrocuring material properties are maintained. Structural activity relationships (SAR) of the blend with respect to its cografter variant is shown in **Figure 5.1**. SAR of blends compared with its cografter variant (between Fc and Dz) lead to the following hypotheses: (1) Ohmic conductivity depends only on ferrocene molar concentration as ferrocene creates the conductive hole network. Higher conductivity leads to faster reaction kinetics. Kinetics are evaluated by gelation time, defined as the time point where $G''/G' = 1$. (2) Carbene generated from diazirine reduction leads to crosslinking. Therefore, crosslinking is strengthened by higher concentrations of diazirine as evidenced by the higher value of storage modulus. (3) The intramolecular Fc-Dz electron transfer in cografter adhesive yields faster gelation time in comparison to blend, which is likely explained by inter- vs. intramolecular electron transfer. This benefit is partially offset by the latter's flexible formulation and ease of synthesis.

Figure 5.1. Schematic illustration of (a) blend electrocuring adhesive with ferrocene and diazirine grafted on G5-PAMAM (b) cograft formulation with ferrocene and diazirine consecutively grafted onto G5-PAMAM.

5.2. Methods

5.2.1. Materials

Poly(amidoamine) (G5-PAMAM, Mw = 28.8 kDa) was purchased from Dendritech, Inc, USA. 4-[3-(Trifluoromethyl)-3H-diazirin-3-yl] benzyl alcohol (hydroxy diazirine) was purchased from TCI, Japan. Chlorocarbonyl ferrocene was purchased from PICHEMICALS, China. Dimethyl sulfoxide (DMSO) and *n*-tetrabutylammonium hexafluorophosphate (*n*Bu₄NPF₆) were purchased from Alfa Aesar, Singapore. Disposable Zensor® chip was purchased from Zensor R&D Company, Taipei, Taiwan.

5.2.2. Conversion of hydroxy diazirine into bromo diazirine

To a solution of hydroxy diazirine (9.3 g, 43.0 mmol) and CBr_4 (16.1 g, 48.6 mmol) in anhydrous CH_2Cl_2 (50 mL) was added PPh_3 (12.7 g, 48.6 mmol) portion-wise over a period of 30 min at 0 °C. The mixture was then warmed to room temperature and stirred at room temperature for 4 h. Hexane was added to the reaction mixture and the precipitate was removed by filtration over celite. The filtrate was evaporated in *vacuo* and the residue was purified by column chromatography on silica gel (diethyl ether/hexane 1:9) to afford the 3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)-3H-diazirine (bromo diazirine) as a colorless oil (10.3 g, 86%).

5.2.3. Synthesis of G5-Fc₁₅ and G5-Dz₁₅ blends

The general procedure given here for the synthesis of **G5-Fc₁₅** and **G5-Dz₁₅** dendrimers was used for the synthesis of G5-Fc₇, G5-Fc₂₂, and G5-Dz₇, G5-Dz₃₀.

G5-Fc₁₅ Synthesis: To a solution of G5-PAMAM (1g, 0.035mmol) in anhydrous methanol (5 mL) was added a methanolic solution (5 mL) of chlorocarbonyl ferrocene (0.34g, 1.33mmol) dropwise under nitrogen atmosphere at room temperature. Triethylamine (0.17g, 1.77mmol) was added and the mixture was stirred for 16 h. The volatile materials were removed under reduced pressure and the residue was stirred vigorously with a mixture of chloroform-Hexane (20 mL, 1:4). The resulting precipitate was filtered and dried to afford G5-Fc₁₅ as an orange solid (0.6 g, 0.018 mmol). This material was used as such for rheological studies without further purification.

G5-Dz₁₅ Synthesis: To a solution of G5-PAMAM (6.96g, 0.035mmol, 14.37% w/v) in anhydrous methanol (8mL) was added a methanolic solution (5 mL) of bromo diazirine (Dz-Br, 0.29g, 0.66mmol) dropwise under nitrogen atmosphere at room temperature. After stirring vigorously for 16 h at room temperature, methanol was removed under reduced pressure. The residue was dried in *vacuo* to afford G5-Dz₁₅ as yellow solid (0.6 g, 0.018 mmol). This material was used as such for rheological studies without further purification.

5.2.4. Synthesis of G5-Fc₇-Dz₁₅ cograft

To a solution of G5-PAMAM (1g, 0.035mmol) in anhydrous methanol (5 mL) was added a methanolic solution (5 mL) of chlorocarbonyl ferrocene (0.18g, 0.66mmol) dropwise under nitrogen atmosphere at room temperature. Triethylamine (0.09g, 0.88mmol) was added and the mixture was stirred for 16 h. The volatile materials were removed under reduced pressure and the residue was stirred vigorously with a mixture of chloroform-Hexane (20 mL, 1:4). The resulting precipitate was filtered and dried to afford G5-Fc₇ as an orange solid (0.60g, 0.019mmol). This material was used as such in the next reaction without further purification. To a solution of G5-Fc₇ (0.60g, 0.019mmol) in anhydrous methanol (8mL) was added a methanolic solution (5 mL) of bromo diazirine (0.10g, 0.38mmol) dropwise under nitrogen atmosphere at room temperature. After stirring vigorously for 16 h at room temperature, methanol was removed under reduced pressure. The residue was dried in vacuo to afford G5-Fc₇-Dz₁₅ as brown solid (0.6 g, 0.016 mmol). This material was used as such for rheological studies without further purification.

5.2.5. NMR spectroscopy analysis of G5-Fc_x and G5-Dz_y

400 MHz Bruker Advance NMR was used to characterize all grafted dendrimers (G5-Fc_x and G5-Dz_y) using DMSO-d₆ solvent. Topspin 3.5pl7 software was applied for both peak assignment and integration of ¹H NMR spectra. The grafting percentage of the attached Fc and Dz functionalities were determined by ¹H NMR. The selected peaks are internal standard peaks (**Appendix B, Figure B1a-b**, peak a), integration of Fc peaks (**Appendix B, Figure B1a**, b1, b2, b3), and integration of Dz peaks (**Appendix B, Figure B1b**, c1, c2).

5.2.6. Size Exclusion Chromatography (SEC) of G5-Fc_x and G5-Dz_y

An Agilent 1100 HPLC size exclusion chromatography (SEC) was used to measure the masses of Fc and Dz grafted G5-PAMAM dendrimers (G5-Fc_x & G5-Dz_y). The masses of grafted dendrimers were calculated from the peak areas detected by the UV detector using Beer-Lambert law. The instrument is equipped with the in-line detectors: UV/Vis, multi-angle light scattering (MALS, Wyatt Technology Corporation, US), and refractive index (RI, Agilent Technologies,

Santa Clara, CA, USA) detectors. PLGel aqueous MIXED-H column in 35 °C oven was utilized in SEC along with 1% w/v formic acid eluent at the flow rate of 1 mL/min. Each of the Fc and Dz grafted dendrimers was dissolved in DMSO-H₂O (2:8) at the concentration of (2 mg/mL), filtered with 0.2µm syringe, and injected (50µL). Dn/dc of 0.185 was used for G5-PAMAM and Fc/Dz grafted dendrimers. The typical elution times are described in **Appendix B, Table B1**, and **B2**, and the UV extinction coefficients are described in **Appendix B, Table B6**.

5.2.7. Cyclic Voltammetry (CV)

PocketSTAT potentiostat (Ivium Technologies, Netherlands) was used to record cyclic voltammograms. The custom-built electrochemical cell was put into a Faraday cage, equipped with a platinum counter electrode, Ag/AgCl reference electrode (filled with saturated LiCl ethanol solution) and glassy carbon (GC) working electrode (3 mm diameter, polished with 1.5, 0.3, and 0.05 µm Al₂O₃). Cyclic voltammetry results were recorded in DMSO solution with 0.1 M *n*-tetrabutylammonium hexafluorophosphate (*n*Bu₄NPF₆) and 15 mM dendrimer (G5-Fc₁₅ and G5-Dz₁₅) concentration at the scan rate of 100 mV/s and were scanned from negative to a positive potential. Three cycles were recorded for the CV of each compound, and the second cycle is presented in **Appendix B, Figure B6**. Cyclic voltammetry of Fc → Fc⁺ (oxidized) and Dz → Dz⁻ (reduced) is presented in **Appendix B, Figure B7**.

5.2.8. Electrorheometry

The Anton Paar MC102 rheometer equipped with a portable potentiostat power supply (pocketSTAT, Ivium Technologies, Netherlands) was used to record viscoelastic properties of the adhesives at -5.0V for 14 mins. A polypropylene-based Zensor® chip, embedded with a 3-mm diameter GC as working electrode (WE), an outer annular crescent GC as the counter electrode (CE), and an Ag/AgCl pellet as a reference electrode (RE), was selected as electrode platform. The oscillatory test was performed at a measuring gap of 0.3 mm, with an amplitude of 10% and frequency of 1 Hz with a rheometer probe parallel plate 5mm in diameter (PP05, Anton PAAR, United States). Each of G5-Fc_x and G5-Dz_y was dissolved in DMSO with 0.1 M *n*Bu₄NPF₆ electrolyte (50% w/w,

sample-DMSO) and mixed well to obtain a homogeneous solution. The blend adhesive was prepared by mixing each of these solutions in an equal ratio (G5-Fc_x-solution and G5-Dz_y-solution, 1:1, w/w). Voltage was applied after two minutes of stabilization under dynamic mechanical analysis and loss modulus (G'') and the storage modulus (G') were recorded with a data sampling frequency of 0.3 Hz.

5.2.9. Lap shear adhesion test

Lap shear adhesion studies were performed according to ASTM standard F2255-05 and the shear adhesive strength failures were performed with TCD110 Series Force Measurement System (Chatillon Force Measurement Products, USA) at the strain rate of 3 mm min⁻¹ with 50 N loading cell. Collagen films were cut into slices (20mm × 20mm × 0.02 mm) and placed onto a glass slide with double-sided tape. A 0.1 mm thick PTFE tape was applied on the zensor electrode as a spacer. An adhesive sample (~20 mg) was put onto the zensor electrode and the slides were fixed with paper clips (pressure applied 27 ± 5 kPa). This system was stimulated for 15 min under -5V using a portable potentiostat (pocketSTAT, Ivium Technologies, Netherlands).

5.2.10. Statistical analysis

All results are exhibited as mean ± SD (where n=3, unless stated otherwise). The significance was evaluated with OriginPro 2018b 64-bit software using one-way ANOVA with Tukey correction as post-hoc test. $p < 0.05$ (*) were considered as statistically significant.

5.3. Results

5.3.1. G5-Fc grafts observe non-specific aggregation but G5-Dz is freely dispersible

¹H NMR quantifies the molar grafting of Fc and Dz with respect to PAMAM-CH₂ peaks (**Appendix B, Figure B1**). The ferrocene grafting through acid chloride intermediates is not straightforward however. Despite employing anhydrous solvents, side reactions (observed at 8.5 ppm, ferrocenecarboxylic acid) lower the grafting yield to 70-80%. SEC coupled to UV/Vis absorbance detectors determine the masses of Fc (440 nm) and Dz (360 nm) grafted onto PAMAM dendrimer. The G5-PAMAM mass is quantified by refractive index (RI) detectors (PAMAM dn/dc = 0.185). This method exploits PAMAM's minuscule UV absorbance at these wavelengths. Grafting Fc and Dz to G5-PAMAM leads to increasing molar mass and a faster elution time in **Figure 5.2a** and **5.2b**. The typical elution times for the blends are listed in **Appendix B, Table B1**, and **B2**. Fc and Dz absorbance elution profiles coincide with G5-PAMAM RI detector and 90° light scattering (data not shown), confirming covalent grafting of Dz and Fc, as display in **Figure 5.2c** and **5.2d**. Higher grafting ratios of G5-Fc lead to aggregation, likely by hydrophobic attraction, π - π stacking, or a combination thereof.[7]·[8]·[9]·[10] This accounts for the limited solubility seen in cograft formulations. NMR and SEC data comparisons in **Appendix B, Table B1** (G5-Dz_y) and **Table B2** (G5-Fc_x) display molar mass, elution time, and calculated grafting ratios.

5.3.2. Scope and structure-activity relationships (SAR) of G5-Fc and G5-Dz blending

Herein, blends of G5-Fc and G5-Dz are compared to the cograft G5-Fc-Dz (G5-PAMAM-Dz-Fc). G5-PAMAM grafted with three different percentages of Dz and Fc are prepared (**Table B3**) following the procedure described earlier and characterized by ¹H NMR and size exclusion chromatography (SEC).[2]·[11] Nine blended formulations (**Table B4**) are then evaluated for SAR studies with the aid of a custom electrorheology instrument. Various material properties of adhesives were determined by the real-time rheology in three timeframes of i)

before curing of 0 V, ii) during voltage activation of -5 V, and iii) after the voltage is removed (**Table B5**). In addition, lap shear adhesion stress at failure evaluates the adhesion strength as a function of Fc/Dz grafting, Fc/Dz ratio, and ultimate elongation.

Figure 5.2. RI analysis, after normalization from SEC-MALS-UV signal measured (a) G5-PAMAM (control) and G5-Dz_y conjugates ($y = 7\%$, 15% , 30%) respectively. (b) G5-PAMAM (control) and G5-Fc_x conjugates ($x = 7\%$, 15% , 22%) respectively. Both are presented in lines. (c) ABS analysis from SEC-MALS-UV signal measured @ 360 nm for G5-PAMAM (control) and G5-Dz_y conjugates ($y = 7\%$, 15% , 30%) respectively. (d) ABS analysis from SEC-MALS-UV signal measured @ 440 nm for G5-PAMAM (control) and G5-Fc_x conjugates ($x = 7\%$, 15% , 22%) respectively. Both are presented in dots.

5.3.3. Fc Grafting Catalyzes the Crosslinking of Electrocurable Adhesive G5-PAMAM, G5-Dz₃₀ and G5-Fc₁₅ shows low to no gelation time within 12 minutes of electrocuring

Electrorheology experimental set-up is illustrated in **Figure 5.3a**. The disposable Zensor electrode energized with a potentiostat @ -5V was optimized in previous investigations.[2] Electrorheology studies investigate viscoelastic properties of the blend before, during, and after voltage activation. Activation of the blend components G5-Fc (donor), G5-Dz (acceptor), or G5-PAMAM alone results in delayed or no electrocuring-based gelation (**Figure 5.3b**). None of the samples formed a thin film ($\tan \delta > 1$, liquid). Even at the highest grafting concentration, no crosslinking occurs when G5-Dz₃₀ or G5-Fc₁₅ exists as a single component.

5.3.4. Controlled concentration formulation of blend and cograft Fc/Dz adhesives provide similar storage modulus and gelation time but different viscosity

Herein, the gelation time, storage modulus, and viscosity of G5-Fc_X (X=22%) and G5-Dz_Y (Y=30%) blends are evaluated with respect to the previously investigated G5-Fc₁₅-Dz₁₅ cograft variant [2] that exhibited liquid properties and crosslinked to a viscoelastic solid within minutes of low voltage activation. **Figure 5.3c** presents a comparative electrocuring results of blend and cograft in controlled concentration with respect to DMSO. As expected both the cograft (gt@2.7 min) and blend (gt@3.0 min) exhibited similar gelation times. However, cograft displayed a slightly higher storage modulus (899 Pa) than blend (555 Pa). This could be attributed to the faster electron mobility in cograft due to the shorter distance between Fc and Dz components than in the blend.[1, 12-14] Further investigation reveals that the blend being the mixture of two PAMAM dendrimers displays higher complex viscosity (10^4 mPa.s) than the cograft variant (10^3 mPa.s) (**Appendix B, Figure B2**). The intermolecular distance of blend displays higher $\tan \delta$ (0.8) while the intramolecular distance of cograft variant displays $\tan \delta$ (0.3) (**Appendix B, Figure B3**). Complex modulus determines the rigidity of the material's viscoelastic structure upon applied stress, both blend and cograft variant display comparable data (10^3 Pa) (**Appendix B, Figure B4**).

Figure 5.3. Real-time dynamic mechanical analysis of, (a) Schematic illustration of the electrorheology experiment setup. (b) G5-PAMAM, G5-Dz₃₀, G5-Fc₁₅. (c) Blend vs Cograft with Fc and Dz controlled concentration comparison. (d) G5-Fc₁₅+G5-Dz_y Blend presents consistent gelation time with different storage modulus. (e) G5-Fc_x+G5-Dz₃₀ Blend presents comparable storage modulus with different gelation time. (f) Summary of Blend Graph with Fc contributes to gelation time. (g) Summary of blend graph with Dz contributes to mechanical properties.

5.3.5. Increasing Fc and Dz grafting exhibits faster gelation time and higher G' , respectively

Figure 5.3d elucidates that gelation time remains consistent while storage modulus increases for the blend containing constant Fc ($x = 15\%$ in G5-Fc_x) and increasing Dz ($y = 7\%-30\%$ in G5-Dz_y) grafting. Contrarily, **Figure 5.3e** elucidates that by keeping constant Dz ($y = 30\%$ in G5-Dz_y) and increasing Fc ($x = 7\%-22\%$ in G5-Fc_x) grafting in the blend the storage modulus remains comparable with faster gelation. **Figure 5.3f** displays gelation time as a function ferrocene percentage in G5-Fc_x ($x = 7\%, 15\%, 22\%$) and **Figure 5.3g** displays storage modulus (G') as a function of diazirine percentage in G5-Dz_y ($y = 7\%, 15\%, 30\%$) in blend. G5-Dz₃₀ control displays $\tan \delta > 1$ without mixing with donor blend G5-Fc_x (**Appendix B, Figure B3a**). This is supported in the amplitude sweep data that shear strain at failure of G5-Dz₃₀ occurs at 3% with the modulus (G') below 10^2 Pa (**Appendix B, Figure B5a**). Addition of G5-Fc₂₂ influences the mechanical property of the blend by achieving higher modulus (G') and shear strain at failure with increased grafting of Dz (**Appendix B, Figure B5b-c**).

5.3.6. Lap shear adhesion correlates to Dz grafting ratio

The lap shear experimental set-up is illustrated in **Figure 5.4a**. Electro curing @ -5V was applied for 15 minutes followed by a lap shear adhesion test with controlled concentration formulation of blend and cogaft. **Figure 5.4b** presents the stress vs strain raw data plots with a constant 22% Fc and varied Dz grafting blends. The adhesion strength at failure displays that adhesion strength increases with the Dz grafting. The G5-PAMAM control in **Figure 5.4c** exhibits low adhesion strength of 10 kPa after 15 mins of electro curing. The area employed (0.28 cm^2) for stress calculations corresponds to the area of working + counter electrode. Correlation of Dz grafting percentage to adhesion strength is attributed to the number of carbenes, which are hypothesized to be the crosslinking moiety. However, the increasing percentage of Fc has minimal effect on the adhesion strength as illustrated in **Figure 5.4c**. Increasing Fc grafting is hypothesized to increase the electron mobility via lowering the tunnel length, which quickens the

gelation time (**Figure 5.3f**). **Figure 5.4c** compare all nine blend formulations with the optimized cograft formulation. The adhesion strength at failure for blend (G5-Dz₃₀+G5-Fc₁₅) and cograft (G5-Fc₇-Dz₁₅) adhesives are 37 kPa and 40 kPa, respectively. The adhesion strength of cograft and blend is comparable due to the Fc/Dz in both adhesives are at a similar concentration. Blends have lower shear viscosity after break than cografts (**Figure 5.4d**).

Figure 5.4. Lap shear adhesion test (a) electrocuring experimental set-up at the interface between collagen film and Zensor® chip electrode. (b) stress as a function of strain for G5-Dz_y+G5-Fc₂₂ adhesives. (c) blend vs cograft formulation in the bar chart. (d) shear stress and shear viscosity after break as a function of strain for G5-Fc₇-Dz₁₅ and G5-Dz_y+G5-Fc₁₅ adhesives.

5.4. Discussion

5.4.1. Fc (Donor) and Dz (Acceptor) grafted blends overcome cografting insolubility

Previous investigation of voltage-activated adhesives (PAMAM-g-diazirine) was limited to aqueous solvents.[1] Operation in hydrophobic environments is required towards translating this technology for structural adhesives in line with advanced manufacturing initiatives. Preliminary trials in organic solvents failed to initiate electrocuring and this was attributed to a lack of electron mobility.[15] Metal-organic frameworks inspired the grafting of donor/acceptor groups, where Fc/Fc⁺ oxidation and Dz/Dz⁺ reduction enhance the charge transport and electron mobility.[16, 17][5] Grafting to G5-PAMAM dendrimers prevents diffusion to the electrodes (on the minute timescale), keeping both donor and acceptor in close proximity. Diazirine doubles as the electron acceptor (forming the diazirinyl radical) and covalent crosslinker (via carbene insertion).[18][19] This dual function is initially advantageous, but as electrocuring progresses the decay of diazirine lowers the electron mobility brought upon by the Dz⁺/Fc⁺ pair. Ferrocene (grafted) serves as an ideal electron donor, as it is stable under ambient conditions and readily oxidized at 0.6 V (vs. Ag/AgCl). A model system of voltage-activated adhesive was then based on ferrocene and diazirine cografted on G5-PAMAM in subsequent syntheses.[20][2] However, the cografting approach is limited to 45% of the available amines on the G5-PAMAM surface—exceeding this created insoluble matrices that would only swell in organic solvents. This severely limited the parameter space to ferrocene and diazirine grafts, with limited grafting availability to overcome the shrinking availability of the Dz/Dz⁺-based crosslinker. Thus, the earlier cograft adhesive was limited to 45% grafting of the total amines on the G5-PAMAM. The formulation library was limited to one grafting concentration of ferrocene (15%) with three diazirine grafting of 7%, 15%, and 30%. Ferrocene's structure-activity relationships to gelation time, modulus, or lap shear adhesion couldn't be evaluated. Herein, this limitation is overcome with nine blended formulations of G5-PAMAM-g-diazirine and G5-PAMAM-g-ferrocene, where the grafting percentage is kept similar to the cograft systems to allow comparison of gelation and crosslinking kinetics.[21]

5.4.2. Hole Network and Enhanced Charge Transport is Retained in Blended Formulations

Ferrocene and diazirine are hypothesized to transform into ferrocenium (Fc^+) and diazirinyl radical ($\text{Dz}^\cdot$) respectively, upon voltages greater than 1.3V (vs. Ag/AgCl, see **Appendix B, Figure B7, B8**). As a result, the donor and acceptor provide a conductive network within the adhesive, presumably activating carbene insertion as the predominant crosslinking mechanism. When Fc and Dz are cografted instead of blended, the intramolecular electron transfer mechanism is more likely due to shorter spacing and shared electronic properties of the dendrimer. Intermolecular electron transfer cannot be entirely ruled out, especially considering the high solute concentrations within these formulations. For cografts, both options are possible and the empirical results at this time cannot govern which is the major pathway. Blended formulations can only rely on intermolecular electron transfer since the transfer from Fc to Dz is physically separated by the dendrimers. Although blending streamlines the design and simplifies the synthesis, electron mobility is impaired compared to cografted dendrimers. For example, peak currents (at 30 ms, first time point) for blended Voltaglue are only 60% of cografted (1.2 vs 2.0 mA). This presents itself in the lap-shear adhesion data (**Figure 5.4d**), where the blended samples have lower viscoelastic properties than cografts. Practically, this means that blended Voltaglue is better when screening redox formulations or investigating SAR. Blended formulations may be further optimized with higher voltages or alternating current for faster activation.[4]·[22]

5.4.3. Fc grafting controls gelation time and diazirine grafting correlates with adhesion

Fc grafting beyond 15% renders the cografts insoluble, preventing SAR investigations.[2] This constraint is overcome by the introduction of Fc-Dz blends wherein Fc grafted up to 22% in G5-Fc_x provides a homogeneous viscous liquid in DMSO. The rheological and lap-shear adhesion studies confirm that an increasing percentage of Fc in blend adhesive only shortens its gelation time without having any impact on the mechanical properties (**Figure 5.3f, 5.3g &**

5.4c). No trends are apparent with respect to Fc grafting on peak current and bulk resistance (**Appendix B, Figure B6**). Herein, some advancements of Voltaglue adhesives in organic solvents are described, but limitations still exist. Mechanical properties are comparably weak of Voltaglue ($G' \sim 1$ kPa) with respect to similar formulations that are photo-activated and are on the order of 100 kPa G' . [23, 24] This stems from the incomplete conversion ($\sim 50\%$ as measured by ^{19}F NMR) of diazirine to the crosslinking group in voltage-activated adhesives. Voltage activation compared to photocuring also appears to have different intermediate pathways to nucleophilic crosslinking. [22]

5.5. Conclusion

Voltage-activated adhesives containing a blend of internally conductive donor-acceptor dendrimer components was developed as a model system for exploring relevant structure-activity relationships. Blended formulations allowed tunable gelation times, adhesion strength, and only require one-step synthesis compared to the two-step cogaft variants. Low voltage activation in non-conductive solvents via donor/acceptor $\text{Fc}^+ - \text{Dz}^-$ networks leads to carbene insertion as the hypothesized crosslinking mechanism. The voltage-activated one-pot blend adhesive presented here can have applications in thermally sensitive and opaque substrates at an ambient temperature, exploring emerging solutions in advanced manufacturing.

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Chapter 6*

Catechol based adhesive using donor (catechol) and acceptor (Schiff base) for electrocuring with the elimination of water electrolysis

Chapter 6 outlines the development of catechol-based voltage-activated adhesives by utilizing the concept of donor-acceptor pairs. Catechol (donor) was grafted onto 5th generation polyamidoamine to form a Schiff base (acceptor). With donor-acceptor pairs, the one-pot catechol adhesive can be voltage-activated well below water electrolysis. This eliminates water electrolysis and nitrogen evolution from carbene based donor-acceptor adhesive system. Hence, it expands the library of voltage-activated adhesives. For the first time, a one-pot catechol adhesive is demonstrated with multiple modes of external activation: self-curing, electrocuring, substrate, and two-part curing. Lap shear adhesion (50 kPa) and viscoelastic properties demonstrate that electrocuring is non-inferior to two-part curing methods.

*This work is published in the following:

Lu Gan[‡], **Nigel C. S. Tan[‡]**, Avi Gupta, Manisha Singh, Oleksandr Pokhonenko, Animesh Ghosh, Zhonghan Zhang, Shuzhou Li, Terry W. J. Steele, Self curing and voltage activated catechol adhesives. *Chemical Communications* **2019**, 5, 10076 -10079. **(First co-author)** (No written permission is necessary for thesis purposes)

6.1 Rationale and Introduction

Hypothesis 4: *Catechol (donor) is oxidized to o-quinone for chemical redox crosslinking. The concept of donor-acceptor voltage-activated adhesion will activate catechol to quinone if a suitable electron acceptor is established within close proximity.*

Catechol-grafted polymers/conjugates are promising hydrogels towards bonding of biomaterials on a variety of surfaces.[1-3] When catechol is oxidized to o-quinone, the latter covalently reacts with amines or itself by a number of mechanisms.[4-6] Although chemical and chelate-mediated crosslinking provides rapid gelation, it requires adding additional initiators that need to be thoroughly mixed. Oxidants,[7, 8] chelating metal ions,[8] and enzymes[9] have been demonstrated to initiate the catechol crosslinking, where all fall under the designation of two-part chemical curing (**Figure 6.1a**). Two-part curing has the advantage of rapid gelation, but this suffers from limited manipulation before application, local cytotoxicity, and narrow pH ranges.[6, 10] Thus, an unmet need exists for a one-pot catechol-mediated adhesive that allows self-curing (with a known lag-time) or through an external stimulus.

Low voltage initiation of Voltaglue bioadhesives has recently been demonstrated on matrices that incorporate donor/acceptor pairs.[11, 12] However, these first proof-of-concept Voltaglue designs required activation of diazirine, whose voltage activation occurs in the range of water electrolysis (-1.6 V vs. -1.23 V, respectively)—imparting significant foaming into the matrix from H₂ and O₂ evolution.[11] Catechols, in comparison, are electrochemically activated well below water electrolysis. Considering the known advantages of catechol redox properties,[13] a design of donor/acceptor pair forms our central hypothesis: dendrimer conjugates that graft catechols as electron/proton donor(s) paired with a reducible acceptor(s) will be voltage-activated for quinone-mediated crosslinking and adhesion. This design would elevate catechols to one-pot adhesives with material properties that are dependent on electric external stimuli.

Figure 6.1. (a) Schematic diagram of catechol being chemically oxidized or electrochemically oxidized into o-quinone, which is responsible to irreversible covalent crosslinking via Michael addition, for example. (b) Synthetic route of 3,4-dihydroxybenzaldehyde (DBA) grafted G5-PAMAM conjugates (G5-DBA_x), with the grafting ratio X = 10%, 20%, and 30%, respectively. (c) Schematic illustration of plausible intramolecular donor/acceptor pair in G5-DBA_x conjugates, which effects the electrocuring performance.

6.2 Methods

6.2.1. Materials

G5-PAMAM dendrimer (5th generation, 28 kDa, 128 primary amines on dendrimer) was supplied by Dendritech, USA. 3,4-dihydroxybenzaldehyde (97%, denoted as DBA) and sodium borohydride (NaBH_4) are supplied by Sigma, Singapore. Disposable TE100 3 electrode chip is purchased from Zensor® R&D Company, Taipei, Taiwan.

6.2.2. Synthesis of G5-DBA_x conjugates (X = 10%, 20%, and 30%)

G5-PAMAM in methanol stock solution is degassed with N_2 prior to any reaction to remove any dissolved O_2 . DBA is dissolved in anhydrous, degassed methanol and then added dropwise to G5-PAMAM stock solution dropwise over a period of 10 min. The reaction was stirred at room temperature under inert gas in dark conditions overnight. Conjugated products are obtained after precipitating in anhydrous diethyl ether, followed by two washes of precipitations with diethyl ether. Residual solvent is removed under vacuum and stored at $-20\text{ }^\circ\text{C}$ no longer than one week.

6.2.3. Synthesis of G5-DBA₂₀ conjugates with reductive amination

Schiff-bases on G5-DBA₂₀ are chemically reduced by reductive amination with 2x eq. of sodium borohydride (NaBH_4) in anhydrous methanol. NaBH_4 solution (1 M) is added dropwise over 5 min and stirred for 1 h. DI water (0.5 mL) is subsequently added and stirred for 1 h to quench excess reducing agent. The reduced products are precipitated and purified as above in anhydrous diethyl ether, re-dissolved into anhydrous methanol, and precipitated again in anhydrous diethyl ether. This process is repeated three more times before drying under vacuum.

6.2.4. UV/Vis characterization

Free DBA and G5-DBA_x were dissolved in methanol and filtered to obtain a clear solution for UV-Vis spectroscopy (UV-Vis-NIR Lambda 950). Reaction kinetics were evaluated with diluted solutions of PAMAM and free DBA in degassed methanol mixed with the designated mol ratio in a 1 cm^3 spectroscopy

cell to obtain the desired conjugation. Once mixed, the reaction mixture was sealed by a cap and placed in the spectrophotometer. The UV spectrum was obtained at certain intervals of time to investigate the reaction kinetics. UV-Vis spectroscopy (NanoDrop 2000 Spectrophotometer, Thermo Scientific™) for reduced G5-DBA₂₀ is characterized in saturated methanol.

6.2.5. SEC-MALLS-UV quantification of catechol units

Size exclusion chromatography (SEC) consists of an Agilent 1100 solvent pump with in-line detectors of 1) UV/Vis detector (280 nm), 2) multi-angle laser light scattering (MALLS), and refractive index (RI) detector for evaluation of catechols, molar mass, and mass detection, respectively. A PLGel aqueous MIX-H column thermostat set at 60°C with 1% w/v formic acid eluent at a flow rate of 1 mL min⁻¹. Conjugates samples are dissolved in eluent at 2-10 mg mL⁻¹, followed by 0.2 µm syringe filtration before injection (50 µL). Dn/dc of 0.185 is applied for PAMAM dendrimers mass detection. The UV extinction coefficient of catechol units are reported in **Appendix C, Figure C7a**.

6.2.6. NMR spectroscopy analysis

Bruker Advance NMR at 400 MHz evaluated the G5-DBA_x and reduced G5-DBA_x in DMSO-d₆ and methanol-d₄ respectively. MestReNova and Topspin software aids the peak assignment and peak integration of ¹H NMR and ¹³C NMR. Conjugates percentage calculation is listed in **Appendix C, Table C2**. 2D NMR is employed to confirm the Schiff-base formation for G5-DBA₂₀ and reduction of G5-DBA₂₀ conjugate.

6.2.7. Cyclic voltammetry (CV)

AUTOLAB with Nova 2.1 software records the cyclic voltammograms. The electrochemical cell equipped with a 3 mm glassy carbon (GC) (or 3 mm Pt disk) working electrode a platinum counter electrode, and an Ag/AgCl reference electrode (filled with 3.0 M KCl solution) are placed in a Faraday cage. GC is pretreated with 1.5, 0.3, and 0.05 µm Al₂O₃ and 1.0 µm diamond polishing powder, followed by rinse through with DI water. The cyclic voltammogram data are recorded in PBS solution at the scan rate of 50 mV s⁻¹. All cyclic

voltammograms are scanned from negative potential to positive potential. CV scanning is performed over three cycles, and the second cycle is reported herein.

6.2.8. Real-time electrorheology and rheology

Dynamic rheometer (MCR102, Anton Paar, Singapore) coupled with a portable potentiostat (Vertex, Ivium Technologies, The Netherlands) activates the voltage potential across the G5-DBA_X samples with the aid of a disposable 3-electrode polypropylene-based Zensor® chip, embedded with a 3-mm diameter GC as working electrode (WE), an outer annular crescent GC as the counter electrode (CE), and an Ag/AgCl pellet as a reference electrode (RE). A ceramic rheometer probe with 10-mm diameter parallel-plate geometry PP10 probe serves as the measuring probe and directly interfaces to the 3-mm diameter WE in contact with 20 µL of the dissolved sample (G5-DBA_X conjugates in PBS solution). The PP10 probe has an optimized 0.30 mm gap size, as gap sizes greater than 0.5 mm were previously found to have excessive gelation times due to diminishing electric field strength.[12] The loss modulus and the storage modulus of the formulations are recorded at 1 Hz strain rate and 1% strain under oscillatory dynamic analysis. The potentiostat was applied to maintain the activation voltage at 0, -1, and +1 V, respectively. The measurement period was controlled within 60 min to avoid the Zensor® chip's insulator coating being dissolved/damaged. Similar parameters are employed for the chemical curing samples with 2 µL NaIO₄ solution (dissolved in PBS in 0.1 mM) added to the 20 µL G5-DBA_X formulations, 5 s of pipet mixing, followed by real-time rheology analysis. For self-curing samples, real-time rheology is initiated after dissolving in PBS buffer. All samples are evaluated in triplicate and minimum torque measurements are 20 nN.m.

6.2.9. Lap shear adhesion of adhesives against collagen films

Collagen film sections (2.5 × 3 cm²) and Zensor® chip are mounted on microscope slides with double-sided tape. G5-DBA_X formulation (20 µL dissolved in PBS) is sandwiched between 3-electrode Zensor® chip and collagen films. A tape of thickness 100 µm serves as a thickness spacer. For the electrocuring samples, -1 V is applied. For the chemical curing samples, 2 µL NaIO₄ solution (dissolved in PBS in 0.1 mM) is mixed for 5 s within the G5-

DBA_x formulation, followed by covering with collagen films. For the control samples, there was no voltage applied, and no NaIO₄ solution added. Lap shear failure is evaluated by a tensile tester fitted with a 50 N force cell (Chatillon Force Measurement Products, USA), with a linear elongation of 3 mm min⁻¹.

6.3 Results

6.3.1. Design rationale of catechol/Schiff base/amine dendrimer conjugates

Previous work on voltage-activated adhesives suggests that donor/acceptor pairs mediate electron transduction, even in a non-aqueous matrix. [12] Catechol is oxidized to reactive intermediates, o-quinone, which are responsible for intermolecular crosslinking. Several catechol small molecules are available with handles for dendrimer grafting. A strategy to simultaneously graft catechol (donor) and an acceptor group would reduce the synthesis to a one-pot reaction. Protocatechuic aldehyde or 3,4-dihydroxybenzaldehyde (DBA) is a naturally derived catechol that spontaneously forms Schiff bases (azomethines) in the presence of amines. Schiff bases are regularly exploited for grafting and are rapidly reduced under aqueous conditions with mild reducing agents (e.g., sodium borohydride in SI Results, **Appendix C**). [14, 15] Simple mixing of DBA with G5-PAMAM (**Figure 6.1b**) grafts both the donor (catechol) and the acceptor (Schiff base) in flawless 1:1 molar ratios to form zwitterionic tautomers that are thermodynamically stable (**Appendix C, Figure C1 and C2**). G5-PAMAM is utilized as the polymer macromolecule, as it offers numerous design advantages. [11, 12, 16, 17] The surface primary amines on the spherical dendrimer limit intramolecular crosslinking while preventing linear entanglements. [18, 19] It is also soluble in both aqueous and organic solvents, unlike chitosan macromers. PAMAM branched polymer is a model system for exploring structure-activity relationships while maintaining an excess of amines for quinone-mediated crosslinking—further modifications are necessary for bioadhesive or underwater applications.

6.3.2. One-pot synthesis produces catechol biomacromolecules with Schiff-base acceptor

Grafting ratios of 10–30% catechol conjugates are explored (G5-DBA_X with X = 10%, 20%, 30%, respectively), but ratios > 44% had limited aqueous solubility (data not shown). Reaction efficiency and final grafting ratios are assessed by ¹H NMR (**Appendix C, Figure C3–C6 and Tables C1, C2**) and size exclusion chromatography (**Appendix C, Figure C7 and Table C2**). Generation of the Schiff base is complete after [8] has been assessed by UV/vis at 405 nm (**Appendix C, Figure C7**). This peak is extinguished after borohydride reduction, which removes the Schiff base acceptor as a control (**Appendix C, Figure C8**). Molar mass of G5-DBA_X correlates positively with the grafting ratio with a reduction of the UV/RI peak elution volume (**Appendix C, Figure C7e**). Redox properties of G5-DBA_X conjugates are examined by cyclic voltammetry (CV) in isotonic PBS electrolyte (**Figure 6.2a and Appendix C, Figure C9**). A catechol-free benzaldehyde is also grafted on G5-PAMAM to serve as a catechol-free control (G5-Ben₂₀, **Appendix C, Figure C10**), and borohydride reduced G5-DBA₂₀ serves as Schiff base free control (reduced G5-DBA₂₀, **Appendix C, Figure C8**). Free DBA (**Appendix C, Figure C9a**) exhibits an irreversible and diffusion-controlled redox behaviour,[20] supported by the following observations: (1) the peak current ratio ($I^{E_{pa0}}/I^{E_{pc0}}$) is less than 1 at the scan rate of 50 mV s⁻¹; (2) both the anodic peaks ($E_{pa0} = 0.52$ V) and the cathodic peaks ($E_{pc0} = -0.02$ V) (vs. Ag/AgCl) increase progressively with the increasing scan rates (v); (3) the plot of peak current as a function of $v^{1/2}$ is linear for both peaks E_{pa0} and E_{pc0} (**Appendix C, Figure C9b**). The successive CV cycles (either use glassy carbon or Pt as a working electrode) exhibited a non-repeatable current signal (**Appendix C, Figure C9c and d**), suggesting that the electrode surface is fouled by electropolymerized DBA film, which in turn inactivates the electrode surface sensitivity.[21, 22] At the scan rate of 50 mV s⁻¹, the cyclic voltammograms of G5-DBA₁₀ retains the irreversible redox behaviour of DBA. G5-DBA₁₀ displays two anodic peaks ($E_{pa1} = 0.29$ V and $E_{pa2} = 0.91$ V) and a minute cathodic peak (**Figure 6.2a**). Scan rate dependent CV confirms the existence of the E_{pa2} peak (**Appendix C, Figure C9e**). The origin of E_{pa2} peak is attributed to the aldehyde/Schiff base, as it is observed in free DBA, G5-Ben₁₀ (E_{pa3} in **Appendix C, Figure C9f**), G5-DBA₁₀, but not in G5-PAMAM (**Figure**

6.2a). All scans of G5-DBA₁₀ and G5-Ben₁₀ are plotted in the first cycle (Appendix C, Figure C9e, and f) since the peak current decreases after the second cycle. E_{pa2} results from the known redox activation of Schiff bases under similar conditions.[23-27]

Figure 6.2. (a) Cyclic voltammograms of G5-PAMAM (as background), G5-Benz₁₀ (as reference), and G5-DBA₁₀ at 50 mV s⁻¹. Electrolyte: PBS (pH 7.2), working electrode: glassy carbon, reference electrode: Ag/AgCl, counter electrode: Pt. Real-time dynamic mechanical analysis of formulation of 30 wt% G5-DBA₂₀ conjugates in PBS electrolyte: (b) two-part curing (mixed with 0.1 mM NaIO₄ solution) and electrocuring (voltage off: 0–2 min; voltage on: 2–48 min, throughout) at -1 V, gt—gelation time. (c) self-curing (no voltage applied and no oxidant added) after rehydration. Storage modulus (G' , solid line) and loss modulus (G'' , dot dashed line). (d) G' values of G5-PAMAM (control) and 30 wt% G5-DBA_x formulation as a function of grafting % initiated by -1V voltage or NaIO₄ oxidant and G5-DBA₂₀

formulation as a function of weight%. 45 wt% formulation is insoluble in PBS. White columns-electrocuring at -1 V, grey columns-two-part curing with periodate.

6.3.3. G5-DBA_x conjugates initiate crosslinking by voltage, but self-curing after a lag time

Real-time rheology is applied to evaluate the mechanical properties (storage modulus- G' and loss modulus- G'') before and after curing stimuli. The customized rheology platform incorporates a ceramic probe to avoid catechol-metal chelates, as aluminum probes/surfaces are found to instantly initiate curing (**Appendix C, Figure C10a**). Voltages of +1 V and -1 V effects on G5-DBA_x formulation's viscoelastic properties, gelation (when $G' = G''$), and lag time (the period before gelation) are compared to a typical two-part curing method (**Figure 6.2b**). Two-part curing with periodate on G5-DBA₂₀ achieves gelation within 3 min and exhibits a kPa shear modulus after 25 minutes. Negative voltage (cathode as WE) displays instantaneous initiation (**Figure 6.2b**), which is removed after the reduction of Schiff base (**Appendix C, Figure C8c**). Voltage initiation rapidly increases G' , with gelation seen at 2.9 min after voltage activation. At 0 V (control), the G5-DBA₂₀ observes self-curing with a lag-time of 25 ± 5 min following aqueous reconstitution (**Figure 6.2c**). Self-curing is found to be tunable with exposure to aerobic environments in a dry state, where oxygen exposure can increase ratios of catechol/quinone (**Appendix C, Figure C1**).

6.3.4. Rapid initiation by -1V suggests Schiff base reduction is the rate-limiting reaction

Rapid initiation of crosslinking (a fast increase of G') is observed at -1V for 15 and 30 wt% formulations (**Figure 6.2d**). High solute (45 wt%) reconstituted formulations are solid aqueous gels but dissolve in methanol (data not shown). When +1 V is applied, a lag time of 2–3 min is noted before crosslinking with a retarded gelation time of 18 min. The voltage polarity simply swaps the working electrode into a cathode (-1 V, acceptor reduction) or anode (+1 V, donor oxidation), where the working/counter electrodes have a surface area ratio of 4/1, respectively. In the catechol free G5-Benz₂₀ formulation, no crosslinking is

observed at either polarity; therefore, crosslinking is solely mediated by the oxidation of catechol (**Appendix C, Figure C10**). However, the Schiff base-free reduced G5-DBA₂₀ loses the ability to be instantly reduced and also has a delayed gelation time of 8.4 min vs. 3 min. Taken together, these observations suggest that reduction of the Schiff base is the rate-limiting reaction under the -1 V conditions, whereby a cathode working electrode facilitates rapid initiation by providing a larger area. Oxygen mediated pathways are responsible for self-curing behaviour, and the +1 V is likely a mixture of both mechanisms. However, minimum grafting of catechol is required before voltage mediated crosslinking is observed, as G5-DBA₁₀ in **Appendix C, Figure C10** displays no gelation within 30 min but can be chemically cured. **Figure 6.2d** compares the G' values of all electrocuring formulations to two-part curing with periodate. G5-DBA₂₀ and G5-DBA₃₀ activation by electrocuring or periodate displayed no significant differences after 60 min.

Figure 6.3. Schematic illustration of: (a) electrocuring of 30 wt% G5-DBA₂₀ formulation with the 3-electrode chip and wet collagen film. Inset: Geometry and effective working area of electrodes on Zensors chip. WE: working electrode, CE: counter electrode, RE: reference electrode. F_N indicates the pulling force and directions. (b) Representative stress/strain plots of 30 wt% G5-DBA₂₀ formulations: self-curing, electrocuring (-1 V), and two-part curing (with 0.1 mM NaIO₄). (c) Lap shear adhesion strength of 30 wt% G5-DBA₂₀ formulation in comparison of self-curing, electrocuring, and two-part curing in < 30 min and > 60 min curing period.

6.3.5. Electrocuring displays higher tack, with equivalent bioadhesive strength to the two-part curing method

Lap shear adhesion is evaluated with wet collagen films, which serve as a mimic of wet tissue substrates.[28] G5-DBA₂₀ at 30 wt% is applied to the collagen and activated with disposable 3-electrode chips (Fig. 3). G5-DBA₂₀ formulation is cured by two methods: electrocuring (-1 V) vs. two-part curing (periodate). Electrocuring at +1V is not evaluated due to the inferior material properties and

gelation time, as shown in the electrorheology analyses. Lap shear adhesion strength at failure evaluates the crosslinked matrix since cohesive failure is seen throughout.

Tack evaluation under 30 min demonstrates that electrocuring G5-DBA₂₀ formulation has a significant increase over two-part curing, and no adhesion is present for the self-curing formulation. The self-curing control sample (atmosphere exposure with neither voltage nor periodate applied) displays only viscous liquid material properties (**Figure 6.3b**). The lap shear adhesion strength comparisons after 60 min are indicated in **Figure 6.3c**. The self-curing formulation has an increase of adhesion strength to $\sim 4 \text{ N cm}^{-2}$ (40 kPa), which is about 4 times higher than 30 min case. Electrocuring continuously for 60 min is non-inferior to two-part curing, which both present $\sim 5 \text{ N cm}^{-2}$ (50 kPa) adhesion strength.

6.4 Discussion

6.4.1. Redox donor/acceptor bioadhesive displays multiple modes of activation

In summary, an azomethine/catechol adhesive is simply synthesized under ambient conditions to yield an acceptor/donor pair. The spontaneous reaction creates a redox-responsive adhesive that can be applied and activated by a number of external stimuli. The inclusion of both electron donor and acceptor groups creates an adhesive that can self-cure after a predictable lag-time. Activation can be triggered under both oxidative and reducing environments. For example, gelation time decreased in the following oxidative conditions: ambient atmosphere, +1 V, or periodate. A reducing environment triggered cross-linking instantaneously, -1 V or aluminum substrates ($\text{Al} - \text{Al}^{3+} + 3\text{e}^-$).

A serendipitous finding was the short-term stability in aqueous solvents, but this allows the development of self-curing adhesion that is semi-stable in a dry state. A bioadhesive that allows liquid manipulation and then self-cures with a known lag-time is a current unmet need for tissue repair. Activation of the lag-time ‘clock’ is as simple as aqueous reconstitution. The lag time of the dried and precipitated formulation drifted over a period of 3 months when exposed to

aerobic environments. This suggests that the lag-time may be optimized in oxygen-free or limited exposure conditions (**Figure 6.2c**). Future work will address tuning lag-time and further shelf-life stability.

6.4.2. Electro curing is non-inferior to two-part curing

For the first time, a catechol-adhesive allows activation by electrocuring, instead of a traditional two-part curing method. This allows activation while avoiding side-effects from oxidation agents or highly concentrated metal chelators. Electro curing adhesive strength was comparable to the periodate two-part curing methods. Two-part curing is a standard method of activation of mussel biomimetic adhesives, with modest adhesion strength of 40 kPa.[8] Polydopamine-co-acrylate cured with periodate achieved up to 70 kPa after one day of curing. Electro curing allows a simpler approach with no additive mixing and more precise control over initiation and gelation time. However, formulations appear to be limited in design—successful electro curing was only observed at 20–30% grafting ratios. Too little (10%) grafting prevented voltage-activation—too much (greater than 40%) displayed aqueous solubility problems that may stem from spontaneous crosslinking under storage. The electro curing formulations herein have some advantages over our previous Voltaglu formulations.[11, 12, 29] This redox donor/acceptor adhesives could be activated at -1V (vs. Ag/AgCl), but Voltaglu formulations need a higher voltage of -1.6 V that evolves gases which can ultimately weaken the adhesive matrix and limit strength. However, Voltaglu appears more stable in oxidative and aqueous environments, as no spontaneous self-curing has been observed under ambient conditions.

6.4.3. Reductive redox processes are the rate-limiting reactions

To our surprise, voltage-activated crosslinking was accelerated when the working electrode was set as the cathode, effectively providing a larger surface area for reductive reactions. This supports the proposed hypothetical acceptor/donor mechanism in **Figure 6.1**, but no direct evidence exists of Schiff base reduction. This is likely due to a combination of factors; G5-PAMAM contains both primary and tertiary amines, providing a local alkaline environment (pH 9–10). If

zwitterionic, the Schiff base catechol would attract electrons to the protonated azomethine (**Appendix C, Figure C1**). At this high pH, protons are limited to the zwitterion or other tautomers known to exist under aqueous conditions (see SI Results, **Appendix C**). A -1 V electrochemical gradient at the working electrode attracts protons from atmosphere O₂-mediated oxidation of catechol, which is thermodynamically favorable (but the internal electronic reduction/oxidation is not, see **Appendix C, Figure C2**). With Schiff bases speculated to be reduced at this -1 V potential, catechol to quinone oxidations is uninhibited. As quinones are responsible for crosslinking, their formation will decrease the time to gelation. Supporting this hypothesis is the empirical result that the aryl-Schiff bases are reduced with sodium borohydride (**Appendix C, Figure C8**), where borohydride anion is estimated to have a formal potential of $E^{\circ} = -0.7$ to -0.43 V.[30]

6.4.4. Comparison of branched polymer-catechol conjugates

Many formulations of chitosan–catechol conjugates have been synthesized—chitosan is a cheap and scalable branched polymer that has been incorporated in many biomaterials. However, chitosan has an inherent solubility limit in acidic mediums that is often addressed with additional grafting. Even with catechol grafting, the functionalized chitosan can only achieve a 6 wt% solution (polymer in aqueous solvent)—which is too dilute for voltage-activation (G5-DBA₂₀ at 10 wt% displays no electrocuring).[6, 31] Grafting 19% to 80% of the total amines with catechol on chitosan displays similar material properties as the reported G5-DBA₂₀ formulations.[15, 32] Others have exploited electrochemical synthesis of chitosan–catechol, where reductive amination grafts chitosan to the surface followed by oxidative activation for catechol–chitosan grafting towards anti-oxidant surfaces.[33] The technology herein may allow a more straightforward approach for similar purposes. G4-PAMAM was grafted with PEG–catechol to form high modulus hydrogels up to 80 kPa,[34] but this required two-part curing with periodate and cure times of 48 hours. Future work will address methods to achieve similar high moduli with reversible, self-curing, and electrocuring properties while building on prior work to achieve in vivo implants and blood biocompatibility.[35-37]

6.5. Conclusion

For the first time, a catechol bioadhesive is synthesized that allows both voltage-activated crosslinking and self-curing properties when reconstituted in the aqueous solvent. The redox-responsive adhesion was made possible by grafting donor (catechol) and acceptor (Schiff base) groups on polyamidoamine dendrimer. The redox bioadhesive was effortlessly synthesized in a one-pot reaction with commercially available reagents. Application is straightforward with shelf-life stability of the dried formulation exceeding a few months. Cathodic electrocuring instantly initiated crosslinking with adhesion strengths that were non-inferior to two-part curing methods, without the complication of additional toxic additives.

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Chapter 7

Conclusions and Recommendations

This chapter provides a thesis summary and suggestions for future work towards the development of donor-acceptor voltage-activated adhesives. Future work is divided into four parts, (i) improve electron transduction efficiency by introducing suitable redox mediators and oligomer/ionic solvents (chapter 4-5), (ii) blends of catechol (donor) and diazirine (acceptor) may improve material properties and optimize crosslinking kinetics of donor-acceptor voltage-activated adhesives to attain mechanical shear modulus of soft tissues (chapter 5-6), (iii) improve electron mobility in organic formulations by mixing with ferrocene blend (chapter 6) (iv) development of catechol variant adhesive to extend the shelf stability of catechol based adhesives (chapter 6).

7.1. Rationale and Introduction

This thesis covers two primary objectives pertaining to advances in electrocuring adhesives: (i) development of donor and acceptor internal additives towards voltage-activated adhesives in organic formulations. Previous investigations demonstrated that voltage-activation was only possible in water formulations. High solvent resistance, limited conductivity, and poor current transmission of organic solvents needed to be overcome; (ii) expansion of the library of donor and acceptor voltage-activated adhesives with oxidative crosslinking (catechol based), with the elimination of water electrolysis and nitrogen gas evolution.

The donor and acceptor voltage-activated adhesive in organic formulation using Fc-Dz pairs (described in Chapter 4) not only overcomes high solvent resistance, it also can be voltage-activated and reach gelation within 60 seconds with no observed water electrolysis. Comparable adhesion strength with previous water-based formulations was observed. Investigation of the structure-activity relationships between donor-acceptor pairs required alternative blend strategies (Fc-Dz), as high grafting ratios of Fc-Dz leads to solubility limits were reached. Fc-Dz cografts required a 2-step synthesis while the blending strategy solved the solubility limit issues and expanded the efficiency of structure-activity relationship (SAR) evaluation studies. This study reveals that increasing Fc (donor) improves gelation time, while increasing Dz (acceptor) improves the strength of the adhesive. It further demonstrates that the Fc-Dz blend adhesives have comparable adhesion strength with the Fc-Dz cogaft adhesives.

The addition of sacrificial oxidants was hypothesized to balance the redox reaction to increase carbene formation via the hypothesized diaziridine oxidation to a carbene—a mechanism based on diaziridine photo-oxidation. Urea peroxide and benzoyl peroxide did not hinder or improve in storage modulus (G'). The storage modulus of the Fc-Dz carbene based adhesive upon voltage activation remains 1 kPa at 0.1% strain [1, 2]. The low modulus may be the consequence of anisotropic moduli, which is supported by the lap shear adhesion strengths of 15-40 kPa [2]. This is comparable to fibrin glue on PLGA/bone substrates [3].

The G5-PAMAM dendrimer prevents entanglement, allowing low viscosity and high solute formulations in photo-activation [4] or voltage activation [5]. However, the storage modulus (G') of dendrimer-based adhesive such as the voltage-activated G5-Fc-Dz carbene-based adhesive is $< 5\%$ of the G' of photocured PAMAM-g-diazirine [4, 6]. It may be due to the incomplete crosslinking from diazirine and plasticization effect from organic solvents such as DMSO, DMF, and PEG400. Introducing suitable redox mediators may further improve electron transduction efficiency and activate more redox-based crosslinking groups.

Electrocuring formulations based on diazirine exhibit nitrogen gas evolution that creates porous structures that ultimately limit the structural integrity of the adhesives. Moving forward from crosslinking based on diazirine reduction, oxidation crosslinking groups were targeted. Catechol's redox properties are electrochemically activated at values below those required for water electrolysis [7]. Voltage activation of donor-acceptor catechol-based adhesive eliminated water electrolysis and gas emission (H_2 , O_2 , N_2) which contributed to higher storage modulus of 3-4 kPa [7]. It also achieved faster gelation time, comparable adhesion strength with the Fc-Dz cogaft/blend adhesive, as well as two-part chemical curing of the catechol adhesive and self-curing ability. Having a catechol adhesive that achieved higher storage modulus combined with the reducing diazirine strategy gives inspiration for two crosslinkers that can be redox balanced, for instance: catechol as a donor (oxidative crosslinking) while diazirine acts as an acceptor (reductive crosslinking) to improve material properties and optimize crosslinking kinetics. Additionally, catechol adhesive can be voltage-activated in aqueous formulations but not in organic formulations. Future work can improve its conductivity by mixing with a ferrocene blend due to the advantage of its reversible redox properties.

A one-pot catechol-based adhesive can be activated through voltage activation and self-curing (with a known lag-time) upon air exposure after mixing with water formulation. This provides adhesion strength > 20 kPa in 60 minutes, but it also limits its storage period after first-time use. Therefore, it is ideal for one-time use to ensure the best performance of the catechol adhesive. A study of

catechol variant, vanillin, shows similar redox properties as catechol in cyclic voltammetry [8]. Vanillin possesses >2 years shelf stability which shows a potential candidate to replace self-curing catechol. Therefore, it is proposed to replace catechol with a variant that has the reactivity of catechol but the stability of vanillin. This will require the screening of suitable compounds with real-time rheology.

7.2. Implications and future suggestions

7.2.1. Introducing suitable redox mediators, oligomer or ionic solvents to the voltage-activated adhesive may improve its electron transduction efficiency and storage modulus respectively

After investigating the structure-activity relationships of donor-acceptor pairs (Fc-Dz), it is observed that increasing the amount of donor (Fc) improves gelation time while increasing the amount of acceptor (Dz) improves mechanical and adhesion properties. However, the storage modulus (G') of the voltage-activated G5-Fc-Dz carbene based adhesive remains at 1 kPa [1, 2]. To increase the mechanical performance of voltage-activated G5-Fc-Dz carbene based adhesive, further suggestions can be implemented by introducing additives such as redox mediators to improve electron transduction efficiency and ionic solvents to improve the crosslinking mechanisms.

There are several electroactive redox mediators which are organic and worth consideration. These include nitroxide radicals and nitroxyl containing polymers, which are used for supercapacitors and organic batteries [9], carbonyl groups incorporated with polymer materials [10], quinone or imide moieties-based polymers such as anthraquinone derivatives and polyimides [11, 12], carbazole units which grafted into polymer backbone for thin films preparation so that to increase the efficiency of electronic and optoelectronic properties [13]. Due to their redox properties, they can be used as additives to create a conductive environment so that there will be an improvement of electron transduction efficiency in voltage-activated adhesives and more crosslinking can occur [14].

On the other hand, incomplete crosslinking upon voltage activation is due to the decreasing current migration over time in **Appendix A, Figure A8**. This leads to the lower G' of voltage-activated adhesives (1 kPa) vs photo-activated adhesives (100 kPa). Even though DMSO has complete miscibility with both aqueous and organic solvents and allows ease of extraction after matrix cross-linking, enabling the possibility of implantable biosensor and current active drug depots, it is also a common plasticizer that contributes to the plasticization effect of the adhesive. Alternative ways to increase storage modulus (G') can be replacing DMSO with ionic solvents [15] or introducing conductive oligomers such as polyaniline (PANI) [16].

Details of mechanical performance and crosslinking kinetics, such as storage modulus (G') and gelation time can be determined by utilizing a real-time electrorheometry setup. The technical challenge would be to dissolve the current formulations with ionic solvents to form a viscoelastic liquid with viscosity less than 10 Pa.s—which is the limit for syringe application. Secondly, different redox mediators such as nitroxyl, quinone, imide, or carbazole units will be mixed with the two liquid adhesives (Fc-Dz conductive oligomer blends and Fc-Dz dendrimer blends) and electrorheology tests conducted at -1 V. Assuming that the redox mediators improve electron transduction efficiency and ionic solvents improve the crosslinking mechanisms, there is a possibility to improve the mechanical performance of donor-acceptor voltage-activated blend adhesive and reach an equivalent mechanical performance of a photoactivated carbene based adhesive (100kPa)[4].

7.2.2. Blends of catechol as donor and diazirine as acceptor may improve material properties and optimize crosslinking kinetics of donor-acceptor voltage-activated adhesives

Both diazirine and catechol have reversible redox properties and can be electrochemically activated by voltage at -1.6 V [5] and -1 V [7] respectively. Since catechol can be voltage-activated well below the voltage required for water electrolysis (-1.5 V), it is possible to apply voltage at -1 V to electrocure a blend of a catechol based adhesive (G5-DBA_x, oxidative crosslinking) which acts as

donor along with a carbene based adhesive (G5-Dz_y, reductive crosslinking) which acts as acceptor and observe the mechanical as well as adhesion performance of the adhesive. When -1 V is applied, catechol grafted onto G5-DBA_x will oxidize to 2 electrons and 2 protons forming o-quinone [17]. At the same time, diazine grafted onto G5-Dz_y will reduce to diazirinyl radicals, followed by covalent crosslinking that is facilitated by carbene insertion mechanisms.

This blending strategy avoids water electrolysis and subsequent foaming. Hence, it will minimize the number of pores created within adhesive and may lead to a higher storage modulus (G'). With the presence of two established crosslinking units from chapters 5 and 6 such as o-quinone from catechol and carbene from diazine in the catechol-diazine blends, this may provide higher crosslinking density. The detailed structure-activity relationship studies can be determined by utilizing a real-time electrorheometry setup as described in Methods. Assuming all o-quinone and carbene are being generated in the blend of G5-DBA_x and G5-Dz_y (highest grafting ratios for x and y, e.g. 30% each) upon voltage activation at -1 V, there are multiple possible sites for o-quinone and carbene to perform intermolecular crosslinking as well as intramolecular crosslinking within G5-DBA_x (Michael addition) and G5-Dz_y (C-H insertion). Hence, there is a possibility to improve the mechanical performance of donor-acceptor voltage-activated blend adhesive and reach a comparable mechanical performance equivalent to a photoactivated carbene based adhesive which has a maximum storage modulus of 100kPa [4].

7.2.3. Voltage-activated catechol adhesive in organic formulation displays poor mechanical performance can be overcome by adding PAMAM-g-ferrocene blend to increase electron mobility and solvent conductivity

A preliminary experiment of voltage-activated catechol adhesive (G5-DBA₃₀) in an organic formulation (DMSO) has been conducted previously. Voltage-activated catechol adhesive (G5-DBA₃₀) in 1xPBS solution exhibits the maximum mechanical performance of 8-9 kPa and lap shear adhesion of 50 kPa

(at 60 min curing). This result presents comparable mechanical performance with the two-part curing of G5-DBA_x with the initiator of NaIO₄[7]. In addition, catechol adhesive (G5-DBA₃₀) in 1xPBS solution also exhibits self-curing in the presence of formation of catechol tautomers in aqueous solution and exposure to oxygen. Therefore, the experiment comprising catechol adhesive in the organic formulation was conducted. However, the same catechol adhesive in the organic formulation (DMSO) displays a relatively low storage modulus of 0.014 kPa but did reach gelation only after voltage activation. (**Figure 7.1**). The limited crosslinking is attributed to limited current conduction—no ions are present on the dendrimer in the organic phase.

Figure 7.1. Real-time electrorheology test on voltage-activated catechol adhesive (G5-DBA₃₀) in an organic formulation (DMSO)

In order to increase the conductivity of the organic formulation based on hypothesis 1, additional either cograf or blend of PAMAM-g-ferrocene may improve electron mobility as it can act as hole transfer carrier/electron donor for the electron to hop to form an immobilized hole network in the organic-based

catechol adhesive system. An immobilized hole network is formed with ferrocene grafted macromolecule to prevent ion migration in the organic solvent.

7.2.4. Development of catechol variant adhesive can extend the short shelf stability of donor-acceptor catechol based adhesive

The adhesive formulation of catechol utilises 3,4 dihydroxy benzaldehyde (DBA) to graft onto G5-PAMAM dendrimer which contains a Schiff base as an acceptor and catechol group as a donor. It exhibits self-curing in the presence of the formation of catechol tautomers in an aqueous solution and exposure of oxygen. A strategy to overcome this situation is to reduce the numbers of catechol groups (two OH groups) into a more stable catechol variant, for instance, vanillin which has one OH group.[8] However, vanillin is resistant to air oxidation, which prevents electrocuring. Vanillin shows similar redox properties as catechol in cyclic voltammetry.[8] Two preliminary experiments of PAMAM grafted vanillin (G5-Vanillin) conducted with no voltage applied and with/without additional of proton accelerator still display no form of gelation and crosslinking (Figure 7.2).

Figure 7.2. A preliminary result of real-time rheology test, (a) 50 min stability of catechol variant adhesive (G5-Vanillin₂₀) in an aqueous formulation (1xPBS) without additional of proton accelerator (ascorbic acid), (b) catechol variant adhesive (G5-Vanillin₂₀) with additional of proton accelerator (0.1M of ascorbic acid)

Based on the data and hypothesis 4 from donor-acceptor catechol based adhesive, further work can replace DBA with a stable catechol variant (vanillin) which is

hypothesized to extend shelf-stability. Different methods of voltage activation are required to cure the stable catechol variant, vanillin. Some suggested examples included (1) applying a positive voltage to oxidize the stable OH group from vanillin followed by negative voltage can initiate electrocuring, (2) addition of proton donor can balance the redox reaction which can initiate electrocuring. To ensure the adhesive has shelf-stability, apparent viscosity and 50 minutes rheology test with no voltage activation will be observed at different periods upon exposure to the atmospheric environment. Structure-activity relationships (SAR) will be characterized by utilizing a rheometer with portable potentiostat as a voltage source for electrocuring. After electrorheology experiment, an amplitude sweep will be conducted to obtain its shear stress at break. The result can address the self-curing issue of one-pot catechol adhesive (G5-DBA_x), prolong shelf-life after exposure to air and resistance to air oxidation.

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Appendix A

Chapter 4: Voltage-activated carbene based adhesives in organic solvent using the concept of donor-acceptor pairs

1. Quantitation of Fc and Dz dendrimer grafting

The conjugation percentages of the attached dual functionalities were estimated by NMR and SEC-MALS-UV techniques individually. The advantage of utilizing NMR is that it possesses highly selective recognition allowing to clearly distinguish Fc and Dz graftings when they cograf on the same dendrimer. While the errors in NMR calculations originate from the selection of internal standard peak (**Figure A1b**, peak s) and the integration of each characteristic peak (**Figure A1b**, peak f₁, f₂, g₁, g₂, and g₃). SEC-MALS-UV excludes the interference from small fragments and grafted Fc and Dz is independently characterized. No residual Dz or Fc precursors were observed in SEC.

The molar mass of cografed conjugates was calculated from RI and MALS analysis, and the grafting % was calculated from UV absorbance analysis.[1] The molar mass of conjugates increased as the grafting of Fc or Dz, leading to the shift of the RI peaks (**Figure A2a**) to smaller elution volumes. The UV absorbance of Dz and Fc in cografed conjugates were assessed independently at 360 nm[1, 2] and 440 nm[3-6] (**Figure A2b-A2c**), respectively. Although both methods have limitations on calculating the conjugation percentage, the consistency of two sets of values and the agreement with the initial molar ratio of reactants indicate that both graftings have been successfully attached on PAMAM dendrimer within the desired grafting percentages.

2. 2D NMR of G5-Fc₁₅-Dz₃₀ analysis displays no detection of Dz and Fc disubstitution on amine

Co-grafting both Dz and Fc to PAMAM surface may result in mono- or di-substituted -NH₂ grafting. ROESY (rotating frame overhauser effect spectroscopy), HMBC (heteronuclear multiple bond correlation), and TOCSY (total correlation spectroscopy) were applied for a qualitative distance determination of Dz and Fc grafting on PAMAM dendrimer. Only the projection

of crossed peaks was displayed on spectroscopy traces. ROESY examines if the protons from Fc and protons from Dz are within 6 Å to have coherent proton coupling. After over 56-hour scanning, G5-Fc₁₅-Dz₃₀ conjugates ROESY results were obtained, as seen in **Figure A3a**: the target cross-peaks ($\delta_{\text{Fc}} = 4.61$ and 4.32 , $\delta_{\text{Dz}} = 7.44$ and 7.19 , ppm) were not shown up (black circles). There is a group of cross peaks ($\delta = 7.44$ vs. 7.19 , ppm) that represents the two groups of protons from aromatic rings. One of the disadvantages of ROESY is that it is affected by the dimension of the detecting species. Since PAMAM is a dendrimer that has 3D dimensions, all the adjacent Fc and Dz grafting could correlation to each other to show the cross-peaks, even if they are not chemically attached to the same primary amines. Hence, ROESY is interfered by 3D dimension structures of conjugates which limits the sample concentration and prolongs the scanning time. TOCSY examines the proton-proton interactions through bonds, which has effect along long distance (5 bonds). It's similar to ROSEY, but is not interfered by 3D dimension structures of conjugates. G5-Fc₁₅-Dz₃₀ conjugates TOCSY results were obtained after 56-hour scanning, as seen in **Figure A3b**. There is no target cross-peaks observed. HMBC is applied to detect the long-range proton-carbon correlations of species and it is not interfered by 3D dimensions of PAMAM. It only shows the correlations for atoms in the same chemical chain. If Fc grafting and Dz grafting was attached on the same $-\text{NH}_2$ group, the target proton and carbon were 4 bonds away. For protons about 2 or 3 bonds away, HMBC can affirm detection, while for protons about 4 and 5 bonds away, this method is less sensitive. After over 56-hour scanning, G5-Fc₁₅-Dz₃₀ conjugates displayed carbons peaks of $\delta = 140 \sim 175$, ppm. Target proton peaks shifting were at $\delta_{\text{Fc}} = 4.61$ and 4.32 , $\delta_{\text{Dz}} = 7.44$ and 7.19 , ppm. While there are no cross-peaks observed.

3. ^{19}F NMR of adhesive formulations before and after electrocuring

Adhesive formulations were retrieved from Zensor® chip after 46-min electrocuring, followed by dissolved in DMSO-d₆ solvent. The electrolyte salt in DMSO (0.1M Bu₄NPF₆) was the internal standard of ^{19}F signals (**Figure A4a**), and aryl diazirine was measured as a reference (**Figure A4b**). All conjugates were dissolved in DMSO electrolyte in 50 wt%. Peaks at -69.21 and -71.10 ppm indicate the PF₆⁻ signal from the electrolyte and -64.68 ppm indicate the $-\text{CF}_3$

signals. CF_3 signals had a 7% decrease in G5-Dz₃₀ formulation before and after electrocuring. By comparison, CF_3 signals in G5-Fc₁₅-Dz₁₅ formulation dropped by 50% when ferrocene is present.

4. Reproducibility of CV scanning for G5-Fc₁₅ conjugates

To examine the reproducibility of CV for G5-Fc₁₅ conjugates, repeated scanings were applied to a representative sample. Background scanings are shown in **Figure A9a**. As can be seen in **Figure A9c**, with increasing scan number, the current intensity decreased gradually. This may be due to the absorbance and deposition of these PAMAM conjugates on WE (i.e., GC) surface that block the electron transfer process between solution and electrodes. Once the WE surface was re-polished by 1.5 μm and 0.3 μm $\alpha\text{-Al}_2\text{O}_3$ particles, the current density increased back to the original value as the 1st scanning. Similar observations for cografted G5-Fc₁₅-Dz_Y conjugates. PAMAM conjugates are considered stable upon CV electrochemistry scanning, and the 2nd scan was reported in CV measurement.

Figure A1. (a) Stacked NMR traces for starting materials (G5-PAMAM) and synthesized conjugates: G5-Fc₁₅, G5-Dz₁₅, and G5-Fc₁₅-Dz₁₅. (b) Representative ^1H NMR of G5-Fc₁₅-Dz₁₅ with peak intensities and peak assignment with MestReNova software.

Figure A2. SEC-MALS-UV signals measured for G5-PAMAM (as control), G5-Dz₃₀ (as control), G5-Fc₁₅ (as control), and G5-Fc₁₅-Dz_Y cografed conjugates. All samples before electrocuring: (a) RI analysis (after normalization). (b) ABS analysis (UV @ 360 nm). (c) ABS analysis (UV @ 440 nm). All samples after 46-min electrocuring: (d) RI analysis (no normalization). (e) and (f) ABS analysis of (UV @ 360 nm).

Figure A3. 2D NMR of G5-Fc₁₅-Dz₃₀ conjugates. (a) ROESY NMR spectra. (b) TOCSY NMR spectra. (c) Upper: target protons and carbons on di-substituted of Fc grafting and Dz grafting on the same surface –NH₂. Lower: HBCN NMR spectra.

Figure A4. Stacked ^{19}F NMR traces for adhesive formulations before and after electrocuring: (a) PF_6^- from 0.1M Bu_4NPF_6 DMSO electrolyte as the internal standard. (b) Free aryl diazirine in 0.1M Bu_4NPF_6 DMSO electrolyte as reference. (c) G5-Dz₃₀ conjugates in 0.1M Bu_4NPF_6 DMSO electrolyte in 50 wt%. (d) Same formulation as (c) that retrieved after 46-min electrocuring. (e) G5-Fc₁₅-Dz₁₅ conjugates in 0.1M Bu_4NPF_6 DMSO electrolyte in 50 wt%. (f) Same formulation as (e) that retrieved after 46-min electrocuring. The intensity of the internal standard was normalized to 1.00.

Figure A5. Real-time dynamic mechanical analysis, where storage modulus (G' , solid line) and loss modulus (G'' , dot dashed line) throughout. (a) G5-PAMAM and G5-Fc₁₅-Dz₃₀ adhesives in DMSO with 50 wt% concentration. (b) G5-Fc₁₅-Dz₁₅ adhesives in DMF and in PEG400 with 25 wt% concentration. Voltage off: 0-2 min. Voltage on: 2-48 min. (c) G5-Fc₁₅-Dz₃₀ adhesives in DMSO with 50 wt% concentration without applying voltage for 40 min. All conjugates formulations contain 0.1 M Bu₄NPF₆ as an electrolyte.

Figure A6. (a) Strong inverse correlation of gelation time vs. Dz grafting percentage. (b) Linear correlations of gelation time vs. sample thickness (black square ■) and G' values at 46 min vs. sample thickness (open circle ○).

Figure A7. (a) G5-Dz₃₀ conjugates and G5-Fc₁₅-Dz_Y electrocuring in the presence of peroxide additives UPO and BPO with fixed molar ratios of conjugates: peroxides = 1: 5. (b) G5-Fc₁₅-Dz₃₀ conjugates electrocuring in the presence of UPO. All organic solvents contain 0.1 M Bu₄NPF₆ electrolyte. All measurements were done in triplicate.

Figure A8. Current (a and c) and resistance (b and d) of formulations measured in electrorheology: solvent blank (as control), G5-PAMAM (as reference), G5-Dz₃₀, G5-Fc₁₅, and G5-Fc_x-Dz_y conjugates.

Figure A9. CV scanings for: (a) DMSO solvent background, DMSO with 0.1 M Bu_4NPF_6 electrolyte background, and 10 mM PAMAM dissolved in electrolyte background. (b) Free chlorocarbonyl ferrocene dissolved in electrolyte with 10 mM concentration. (c) Representative CV scanings for G5- Fc_{15} conjugates sample dissolved in an electrolyte with 10 mM concentration.

a) Aryl diazirine / G5-Dz₁₅ quasi-reversible in aqueous

b) Fc graftings in G5-Fc₁₅-Dz_Y quasi-reversible in organic

Figure A10. Electron transfer paths for diazirine and ferrocene. [R], reduction. [O], oxidation. (a) Aryl diazirine and G5-Dz₁₅ quasi-reversible redox behaviour. In the absence of a proton donor (e.g. acetic acid) the diazirinyl radical has a half-life of 46 s at 10°C.⁷ (b) Fc graftings in G5-Fc₁₅-Dz_Y quasi-reversible redox behaviour in the absence of acetic acid in an organic solvent. V_a indicates the anodic potential, and V_c indicates the cathodic potential.

Figure A11. UV absorbance spectra of PAMAM (as control), G5-Fc₁₅ (as reference), and G5-Fc₁₅-Dz₁₅.

Figure A12. Energy diagram of Dz and Fc in conjugates. HOMO = highest occupied molecular orbital, LUMO = lowest unoccupied molecular orbital. $E(\text{HOMO}) = -[E_{\text{ox}} + E_{\text{ref vs NHE}}]$ eV, $E(\text{LUMO}) = -[E_{\text{red}} + E_{\text{ref vs NHE}}]$ eV, $E_{\text{ref vs NHE}} = 4.5$ (normal hydrogen electrode) - 0.205 (Ag/AgCl reference electrode in 3.5 M KCl at 25°C) eV = 4.295 eV.

Table A1. Conjugation percentages of G5-Fc_X and G5-Fc_X-Dz_Y obtained from NMR and SEC-MALS-UV in comparison to expected values from Fc/G5-PAMAM and Dz/G5-Fc₁₅ initial molar ratio applied in the synthesis reaction.

theoretical conjugations [%]		conjugation		conjugation		PDI (std)	molecular weight (kDa)
		calculated from		calculated from			
		NMR		SEC-MALS-UV			
		[%]		[%]			
G5-		--	--	--	--	1.06	30 ± 0.6
PAMAM						(3%)	
G5-Fc _X -Dz _Y							
Fc	Dz	Fc	Dz	Fc	Dz		
(X)	(Y)	(X)	(Y)	(X)	(Y)		
15	0	17.9 ±	0	13.8 ±	0	1.11	35 ± 1.4
		0.7		1.0		(5%)	
	7	13.1 ±	7.2 ±	12.0 ±	7.6 ±	1.64	36 ± 1.4
		0.2	0.6	0.9	0.2	(6%)	
	15	17.2 ±	16.5 ±	18.7 ±	18.7 ±	1.68	38 ± 1.9
		1.3	1.9	1.4	0.4	(7%)	
	30	12.3 ±	30.5 ±	14.8 ±	38.3 ±	1.87	45 ± 8.5
		2.7	0.5	2.8	7.0	(23%)	

Table A2. Parameters obtained from cyclic voltammetry measurements. Scan rate at 100 mV s⁻¹. E_{pa} = oxidation peak potential, E_{pc} = reduction peak potential, ΔE_p = peak separation = $E_{pa} - E_{pc}$. Fc = ferrocene, Dz = diazirine, NA = not applicable, ND = not determined. † = no acid in DMSO solvent.

	scan					
	window	E_{pa} (V)		E_{pc} (V)		ΔE_p (V)
	(V)	no acid	with acid	no acid	with acid	no acid
aryl diazirine	-2.5 ~ -0.5	-1.00	N.A.	-1.60	-1.65	0.60
G5-Dz ₁₅	-2.0 ~ -0.5	-1.10	N.A.	-1.25	-1.30	0.15
		Fc	Dz	Fc	Dz	Fc
free Fc [†]	-0.5 ~ 0.8	0.33	NA	0.05	NA	0.28
G5-Fc ₁₅ [†]	-0.7 ~ 1.5	0.63	NA	0.47	NA	0.16
G5-Fc ₁₅ -Dz ₇ [†]		0.61	ND	0.38	ND	0.23
G5-Fc ₁₅ -Dz ₁₅ [†]	-2.0 ~ 1.0	0.63	ND	0.40	-1.30	0.23
G5-Fc ₁₅ -Dz ₃₀ [†]		0.74	ND	0.34	-1.30	0.40

Table A3. Summary of the UV coefficient values for ferrocene (Fc) and diazirine (Dz) graftings.

wavelength	UV extinction coefficient (mL cm ⁻¹ g ⁻¹)	
	360 nm	440 nm
G5-PAMAM	---	---
Dz	1009.5	---
Fc	443	235

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Appendix B

Chapter 5: Structure-Activity relationships of cograft vs blends Fc and Dz dendrimer-based adhesives in organic solvent

Table B1. diazirine mass conjugation on G5-Dz_y (@360nm)

GPC and NMR comparison of Dz grafting onto G5-PAMAM.					
	theoretical [%]	SEC- MAL-UV [%]	NMR [%]	M _w (kDa)	Elution time [min]
G5- PAMAM				29 ± 1	8.51
G5-Dz _y					
Y =	7%	5 ± 2	6 ± 2	38 ± 2	8.49
	15%	14 ± 1	15 ± 2	48 ± 1	8.48
	30%	30 ± 2	28 ± 1	49 ± 6	8.47

*second column: theoretical conjugations [%]
 conjugation calculated from SEC-MALS-UV [%]
 conjugation calculated from NMR [%]
 weight-average molar mass (kDa)

Table B2. Ferrocene mass conjugation on G5-Fc_x (@440nm)

GPC and NMR comparison of Fc grafting onto G5-PAMAM.					
	theoretical [%]	SEC- MALIS-UV [%]	NMR [%]	M _w (kDa)	Elution time [min]
G5- PAMAM				29 ± 1	8.51
G5-Fc _x					
X =	7%	6 ± 1	8 ± 2	47 ± 2	8.48
	15%	13 ± 2	16 ± 2	104 ± 2	8.47
	22%	23 ± 2	22 ± 1	181 ± 1	8.46

*second column: theoretical conjugations [%]
 conjugation calculated from SEC-MALS-UV [%]
 conjugation calculated from NMR [%]
 weight-average molar mass (kDa)

Table B3. Molar mass ratio of G5-PAMAM surface groups (NH₂), Fc and Dz.

[mol]	[NH ₂]	[Fc]	[Dz]
Molar /g	4.44 x 10 ⁻³	7% = 6.66 x 10 ⁻⁴ 15% = 1.33 x 10 ⁻³ 22% = 2.66 x 10 ⁻³	7% = 3.11 x 10 ⁻⁴ 15% = 6.66 x 10 ⁻⁴ 30% = 1.33 x 10 ⁻³

Table B4. Storage modulus of -5V curing Fc-Dz blend samples in 50% weight ratio concentration

Samples	$\tan\delta$ (loss factor)	Gelation time [min]	Storage Modulus [Pa] <i>After curing at 12 mins</i>
G5-PAMAM	>100	no gelation	<1 Pa
G5-Dz ₃₀	1.02	no gelation	18 ($\pm 5\%$)
G5-Fc ₁₅	1.33	no gelation	49 ($\pm 3\%$)
G5-Fc ₀₇ +G5-Dz ₀₇	0.94	9.8	200 ($\pm 5\%$)
G5-Fc ₀₇ +G5-Dz ₁₅	0.82	8.7	209 ($\pm 5\%$)
G5-Fc ₀₇ +G5-Dz ₃₀	0.93	8.7	556 ($\pm 3\%$)
G5-Fc ₁₅ +G5-Dz ₀₇	0.94	5.9	189 ($\pm 3\%$)
G5-Fc ₁₅ +G5-Dz ₁₅	0.77	5.3	219 ($\pm 5\%$)
G5-Fc ₁₅ +G5-Dz ₃₀	0.77	5.4	525 ($\pm 2\%$)
G5-Fc ₂₂ +G5-Dz ₀₇	0.88	3.7	289 ($\pm 5\%$)
G5-Fc ₂₂ +G5-Dz ₁₅	0.80	3.1	315 ($\pm 3\%$)
G5-Fc ₂₂ +G5-Dz ₃₀	0.90	3.0	555 ($\pm 2\%$)

* 50% sample to solvent weight ratio activated at -5V in DMSO

Table B5. Real-time rheology in three regions of timeframes

Region	Time (minutes)	Analysis and measurement
1	0-2	dynamic viscosity (G'' /angular freq) (@ 0.0V)
2	2-14	G'' (loss modulus) and G' (storage modulus) (@ -5.0V)
3	14-15	amplitude sweep (for selected samples) where G'' and G' displayed as a function of strain. This calculated G' , yield stress, and linear viscoelastic region (LVR).

Table B6. UV coefficient values for G5-PAMAM, Fc, and Dz graftings

	UV extinction coefficient ($\text{mL cm}^{-1} \text{g}^{-1}$)	
wavelength	360nm	440nm
G5-PAMAM	---	---
Fc	443	235
Dz	1009.5	---

Figure B1. a) ^1H NMR spectrum for G5-PAMAM control, G5-Fc₇₋₂₂, b) ^1H NMR spectrum for PAMAM control, G5-Dz₇₋₃₀

Figure B2. Complex viscosity (η^*) comparison where electrocuring starts at 2 minutes, a) controls, b) blend vs cograft with Fc and Dz controlled concentration, c) G5-Fc₁₅+G5-Dz_y blend, d) G5-Dz₃₀+G5-Fc_x blend

Figure B3. Tan Delta (δ) comparison where electrocuring starts at 2 minutes, a) controls, b) blend vs cograft with Fc and Dz controlled concentration, c) G5-Fc₁₅+G5-Dz_y blend, d) G5-Dz₃₀+G5-Fc_x blend

Figure B4. Complex Modulus comparison where electrocuring starts at 2 minutes, a) controls, b) blend vs cograft with Fc and Dz controlled concentration, c) G5-Fc₁₅+G5-Dz_y blend, d) G5-Dz₃₀+G5-Fc_x blend

Figure B5. Amplitude Sweep (G^*) comparison, a) G5-Dz control, b) G5-Fc₂₂ + G5-Dz₀₇ blends, c) G5-Fc₂₂ + G5-Dz₁₅ blends, d) G5-Fc₂₂ + G5-Dz₃₀ blends

*G5-PAMAM and G5-Fc₂₂ were not cured

Figure B6. Current as a function of time during electrocuring, a) G5-PAMAM, G5-Dz₃₀ and G5-Fc₁₅ controls, b) G5-Fc₁₅ + G5-Dz_y blends, c) G5-Fc₂₂ + G5-Dz_y blends, d) G5-Fc_x + G5-Dz₃₀ blends, resistance as a function of time for e) G5-Fc₂₂ + G5-Dz_y blends, f) G5-Fc_x + G5-Dz₃₀ blends, where x = 7, 15, 22% and y = 7, 15, 30% (current begins to saturate after gelation is reached for Figure B6b-d)

Figure B7. Cyclic voltammetry data comparison were recorded in DMSO containing 0.1 M Bu_4NPF_6 as electrolyte utilizing working electrode (d = 3 mm): glassy carbon, reference electrode: Ag/AgCl, counter electrode: Pt. Scan rate: 100 mV/s, a) G5-PAMAM control, b) G5-Fc₁₅ ($E_{\text{pa}} = 0.63$, $E_{\text{pc}} = 0.37$), c) G5-Dz₁₅ ($E_{\text{pa}} = -1.10$, $E_{\text{pc}} = -1.25$)

Figure B8. Cyclic voltammetry comparison, a) G5-Fc to G5-Fc⁺, b) G5-Dz to G5-Dz⁻

Appendix C

Chapter 6: Catechol based adhesive using donor (catechol) and acceptor (Schiff base) for electrocuring with the elimination of water electrolysis

Known pKa and tautomers of aryl-aldehyde Schiff-bases

The Schiff-base catechol (phenolic tautomer, **Figure C1**) predominates in non-aqueous solvents like methanol employed in the synthesis, which displays similar UV/Vis peaks to the aryl-aldehyde Schiff-base base formed from salicylaldehyde and alkyl-amines[1-3]. The semiquinone tautomer (**Figure C1a**, extrapolated from aryl-aldehyde Schiff-base) primarily exists in aqueous solutions (tautomer ratio of 0.088) and is similar in structure to quinone methides that are known to self-polymerize[4, 5]. Extrapolated pKa values from substituted catechols (e.g. ethyl catechol, $pK_{a1} = 8.32$) and aryl-aldehyde Schiff-bases (pK_a ranging from 11-12) advocate that the Schiff-base catechol is in a zwitterionic state at the pH's of 8.5-11. Atmospheric oxygen is known to induce free radicals on catechols and spontaneously from quinones, as shown in **Figure C1b**[4]. The hypothesized electrocuring method at -1 V is given in **Figure C1c**.

Thermodynamic modelling of Schiff-base catechol to reduction intermediate

Density functional theory (DFT) calculations[6] on the catechol molecule and its potential reaction intermediate are calculated in **Figure C2**. All calculations of geometry optimization, vibrational frequency analysis, wavefunction stability analysis, and single point energy were conducted via the B3LYP method[7, 8] with 6-311G(d,p)[9, 10] basis set and D3 dispersion correction under Becke-Jonson damping[11]. The Gibbs free energy, which is the difference between the ground energy of the intermediate and the ground energy of catechol is calculated to be 1.4018 kJ/mol. This small energy barrier suggests an unspontaneous reaction between these two states thermodynamically.

Quantitation of DBA grafting by ^1H NMR before and after reductive amination

DBA ^1H and ^{13}C NMR peaks are indicated in **Table S1**. As for the conjugates (i.e., G5-DBA_x), the expected characteristic signals come from: (1) azomethine ($\text{H}-\text{N}=\text{C}-$) group, as seen at 8.02 ppm overlaps with exchangeable $-\text{H}$ atoms ($-\text{NH}$) from PAMAM, preventing integration, (2) $-\text{OH}$ groups in DBA is obscured in the conjugate at 7.85-7.98 ppm, (3) aromatic protons at 7.21-7.08 ppm, 6.98-6.85 ppm, and 6.76-6.63 ppm, respectively, which can be the representative of catechol group. As for the internal standard, $-\text{CH}_2-\text{CO}-$ in PAMAM structure provides proton signal at 2.30-1.90 ppm, which is calibrated as 504 $-\text{H}$ atoms. Hence, the DBA/PAMAM molar ratio (i.e., grafting ratio) is calculated by the following method.

The number of type ‘g’ protons in G5-PAMAM = 504. The number of type ‘b’ or ‘c’ or ‘d’ protons in DBA = 1. I_g — the intensity of peak ‘g’, I_b — the intensity of peak ‘b’, I_c — the intensity of peak ‘c’, I_d — the intensity of peak ‘d’.

Conjugation percentage of DBA (%) = $\frac{(I_b+I_c+I_d/3)}{I_g/504} \times 100\%$. For example, in G5-DBA₅₀ (**Figure C3**), I_g is assigned 504 protons/PAMAM, and integration of I_b , I_c , and I_d yields 53.4, 53.9, and 54.1 protons/PAMAM, respectively. After averaging, a conjugation percentage of 53.8% is calculated. 2D NMR, heteronuclear single quantum coherence (HSQC) spectrum is applied to confirm the presence of the Schiff-based before (**Figure C4**) and after (**Figure C6**) reduction amination. G5-DBA₂₀ conjugate contains a cross-peak of the signal at 8.02 ppm $\times$ 162.33 ppm, which is attributed to the imine coupling. The zoom-in spectrum displays both the imine (blue circle) and catechol protons (green circle). After reductive amination, no ^{13}C peak is observed near 162 ppm (**Figure C5**) and further analysis with 2D NMR HSQC (**Figure C6**) observes no peak in the general region, suggesting little to no Schiff-bases remain after treatment by the NaBH_4 reducing agent.

Quantitation of DBA grafting and molar mass analysis

Catechol quantitation (**Figure C7a-C7c**), Schiff-base reaction kinetics in (**Figure C7c-C7d**), SEC (**Figure C7e**), and extended stress-strain plots (**Figure**

C7f) are displayed. Absorbance (ABS) at $\lambda_{280\text{nm}}$ is attributed to -OH auxochrome on DBA units which is the characteristic ABS peak of catechol species (**Figure C7b**) gives 3 peaks at 230, 280, and 310 nm. A new peak at 405 nm is attributed to the Schiff base (-N=CH- ϕ) coupling. UV extinction coefficient value (**Figure C7a**, $\epsilon_{280\text{nm}} = 14556 \text{ g}^{-1}.\text{mL}.\text{cm}^{-1}$) of catechol was obtained via Beer-Lambert Law. The absorption spectrum displays similar absorption peaks (280, 316, 405 nm) to the previously investigated system of salicylaldehyde and alkylamines (285, 320, 415 nm), which has a predominant phenolic tautomer in methanol [1, 2]. The higher grafting ratio of G5-DBA_x took a longer time to reach equilibrium, where the equilibrium $\log K_f$ of 2-4 drives the reaction to completion, albeit slowly[1, 3]. To achieve more than 75% conversion of Schiff-base formation, grafting ratios took 71 min, 153 min, and 160 min, for G5-DBA₁₀, G5-DBA₂₀, and G5-DBA₃₀, respectively. Reaction efficiency and final grafting ratios were assessed by SEC and results are displayed in **Figure C7e**. With the known $\epsilon_{280\text{nm}}$, the grafting ratio is quantified, and molar mass determination is listed in **Table C2**. As is seen in **Figure C7e**, the molar mass of G5-DBA_x positively correlates with the grafting ratio, leading to the shift of UV peaks at $\lambda_{280\text{nm}}$ and RI peaks to smaller elution volumes.

Assessment of G5-DBA₂₀ before and after reductive amination. The G5-DBA₂₀ before after reductive amination is compared by UV/Vis wavelength scan. After reduction of the Schiff-base to the secondary amine, the peak at 405 nm is extinguished (**Figure C8a**). The reduced G5-DBA₂₀ is compared via self-curing (**Figure C8b**), -1 V electrocuring (**Figure C8c**), or +1 V electrocuring (**Figure C8d**). No changes are observed for self-curing of +1 V electrocuring. However, instantaneous activation via reductive electrocuring (-1 V) is no longer present, and the gelation time is retarded from 3 to 8 min as compared with DBA₂₀ conjugate. However, the gelation time is still faster than +1 V, and this is speculated to be voltage-activated electrocuring via the tautomerized quinone-methide[4, 12].

Cyclic voltammetry analyses of DBA, G5-DBA₁₀, and G5-Benz₁₀

DBA is subjected to a number of scan rates and two kinds of working electrodes (**Figure C9a-C9d**), displaying irreversible redox behaviour. The behaviour is

retained when DBA is grafted to G5-PAMAM via a Schiff-base coupling (**Figure C9e**). Synthesis of the non-catechol Schiff-base coupling of G5-Benz₁₀ observes no oxidation peak from 0.3-0.4 (**Figure C9f**), suggesting the Schiff-base is redox-active even in the absence of catechol, and it responsible for E_{pa2} in **Figure C9e**.

Redox curing of G5-DBA₁₀, G5-DBA₂₀, and G5-Benz₂₀

G5-DBA₂₀ was observed to instantly activate upon contact with aluminium surfaces (**Figure C10a**), which precluded the employment of metal probes on the rheometer. Catechols are known to form chelate complexes with Al(III) and metal oxides that are present on aluminium surfaces such as the disposable aluminium probes^[13]. This necessitated non-conductive, 10 mm ceramic probes, which covered the working and counter electrodes of the 3-electrode disposable chip (see **Figure 6.3a**). Despite high solute concentrations, low grafting ratios of G5-DBA₁₀ did not under electrocuring (**Figure C10b**), but are capable of chemical curing with periodate (**Figure C10c**), suggesting that electrocuring is dependent on intramolecular dendrimer spacing. If no catechol is present, gelation is not observed under self-curing, -1 V, or 1 V electrocuring (**Figure C10d-C10f**) despite high solute concentrations of G5-Benz₂₀.

Table C1. Peak assignments of target conjugates.

compound		chemical shift (ppm)
$C_6H_5O_2^-$ $C(=X)-H$	-O-H	H(β')	H(β)	H(α)
DBA where X = O	9.71	10.09 (s,1H) 9.53 (s,1H)	7.25 (d,1H)	7.28 (dd,1H)	6.92 (d,1H)
G5-DBA where X= N- PAMAM	8.02	coalescent with other exchangeable acidic H atoms	7.15 (s,1H)	6.92 (d,1H); 6.70 (d,1H)	

Table C2. NMR and SEC-MALLS-UV comparison of DBA grafting onto G5-PAMAM.

	theoretical conjugations [%]	conjugation calculated from NMR [%]	conjugation calculated from SEC- MALLS-UV [%]	PDI (std)	molecular weight (kDa)
G5- PAMAM				1.04 (0.8%)	30.5 ± 0.2
DBA _X - PAMAM					
X =	10%	11 ± 0.5	9.9 ± 0.1	1.05 (0.8%)	32.9 ± 0.3
	20%	23.6 ± 0.7	25.1 ± 0.7	1.31 (1%)	35.1 ± 0.7
	30%	28.9 ± 0.3	29.6 ± 0.4	1.21 (1%)	36.8 ± 1.1

Figure C1. (a) Extrapolated tautomers of aromatic aldehydes and ionic structures of Schiff-base catechol based on the known $pK_a[CT]$ of substituted catechol (8.3-9) and $pK_a[SB]$ of aromatic Schiff-base (11-12). [1, 14]. (b) Postulated mechanism of oxygen induced quinone formation. [4, 5]. (c) Hypothesized mechanism of -1 V electrocuring with the protonated azomethine as an electron acceptor.

Figure C2. Density functional theory (DFT) calculations on the catechol and its tautomer intermediate have been performed to gauge if the reaction is thermodynamically favourable.

Figure C3. Stacked ^1H NMR traces for starting materials (3,4-DBA and G5-PAMAM) and synthesized conjugates: G5-DBA₁₀ and G5-DBA₅₀. Peak intensities and peak assignment are analysed with MestReNova software.

Figure C4. 2D heteronuclear single quantum correlation (HSQC) NMR spectrum of G5-DBA₂₀: (a) Full range spectrum. (b) Zoom-in spectrum, where the cross peak at 8.02 ppm $\times$ 162.33 ppm proves the proton at 8.02 ppm belongs to C-H group in azomethine (H-N=C-) linker.

Figure C5. ^1H and ^{13}C NMR traces for G5-DBA₂₀ with reductive amination. Peak intensities and peak assignment are analysed with Topspin software. a' integration is set to 504. The sum of b', c', and d' is 48 or an average grafting ratio of 16% as DBA:PAMAM.

2D NMR G5-DBA₂₀ reduced

Figure C6. 2D heteronuclear single quantum correlation (HSQC) NMR spectrum of G5-DBA₂₀ with reductive animation (a) Full range spectrum. (b) Zoom-in spectrum, where the cross peak at 8.02 ppm $\times$ 162.33 ppm shows no presence of **H**-N=C- linker.

Figure C7. UV extinction coefficient at 280 nm values determined by UV/Vis spectrum of 3,4-DBA: (a) Absorbance as a function of concentration. (b) Absorbance as a function of wavelength. (c) Kinetics plots of DBA grafting reactions, absorbance as a function of wavelength. (d) Kinetics plots of DBA_x grafting reactions, absorbance (at $\lambda_{405 \text{ nm}}$) as a function of time, R^2 value for G5-DBA_{10,20,30} = 0.997, 0.996, 0.996 respectively. Solid lines indicate the Exponential ExpDecay fitting lines. (e) Size exclusion chromatography of G5-PAMAM and G5-DBA_x conjugates Refractive index (RI, normalized) in solid lines, UV detector (at $\lambda_{280 \text{ nm}}$) in dashed lines. (f) Representative stress/strain plots of 30 wt% G5-DBA₂₀ formulations: self-curing, electrocuring (-1 V), and two-part curing (with 0.1 mM NaIO₄) for measurement > 60 mins.

Figure C8. UV/Vis spectroscopy and Real-time rheology test before and after reductive animation comparison: (a) UV/Vis comparison between G5-DBA₂₀ (red line) and G5-DBA₂₀ reduced (black line), where no present of Schiff base UV peak at 405 nm after reduction (b) 30 wt% G5-DBA₂₀ and G5-DBA₂₀ reduced at self-curing. (c) 30wt% G5-DBA₂₀ and G5-DBA₂₀ reduced, (voltage off: 0–2min; voltage on: 2–30 min), gelation time (gt) at 3 min and 8.4 min for -1 V electrocuring respectively. (d) 30 wt% G5-DBA₂₀ and G5-DBA₂₀ reduced (voltage off: 0–2min; voltage on: 2–30 min), gelation time (gt) at 18 min and 18.1 min for +1 V electrocuring respectively. All formulations were prepared in PBS for (b), (c), and (d).

Figure C9. CV of free 3,4-DBA: (a) Scan rate dependent test from 50 to 200 mV s⁻¹. (b) I_p increase depends on the square root of scan rate. (c) Repeated scanned by glassy carbon (GC) working electrode. (d) Repeated scanned by Pt disk working electrode. CV of G5-DBA₁₀: (e) Scan rate dependent test from 10 to 200 mVs⁻¹. CV of G5-Ben₁₀ conjugates: (f) Scan rate dependent test from 10 to 100 mV s⁻¹. Electrolyte: PBS (pH 7.2), working electrode: glassy carbon (except (d)), reference electrode: Ag/AgCl, counter electrode: Pt.

Figure C10. Real-time rheology test: (a) 30 wt% G5-DBA₂₀ at -1 V electrocuring utilizing PP05 Aluminum disposable probe, sample loading takes 60s before starting measurement. (b) 30 wt% and 45wt% G5-DBA₁₀ at -1 V electrocuring. (c) 30 wt% and 45 wt% G5-DBA₁₀ at two-part curing with 0.1mM NaIO₄. (d) 30 wt% G5-Benz₂₀ at self-curing. (e) 30wt% G5-Benz₂₀ at -1 V electrocuring. (f) 30 wt% G5-Benz₂₀ at +1 V electrocuring. All formulations were prepared in PBS.

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