LETTER

Highly Adhesive Phenolic Compounds as Interfacial Primers for Bone Fracture Fixations

Axel Nordberg,[†,‡] Per Antoni,[‡] Maria I. Montañez,[‡] Anders Hult,[‡] Hans Von Holst,[*,†] and Michael Malkoch[*,‡]

Division of Neuronic Engineering, School of Technology and Health, Royal Institute of Technology, Alfred Nobels Allé 10, SE-14152 Huddinge, Sweden, and Division of Coating Technology, Fibre and Polymer Technology, Royal Institute of Technology, Teknikringen 56–58, SE-10044 Stockholm, Sweden

ABSTRACT Bone fractures are today stabilized with screws and metal plates. More complicated fractures require alternative treatments that exclude harsh surgical conditions. By adapting the benign and UV initiated thiol-ene reaction, we efficiently fabricated triazine-based, fiber-reinforced adhesive patches within 2 s. To enhance their bone adhesion properties, we found that a pre-treatment step of bone surfaces with phenolic dopamine and poly(parahydroxystyrene) compounds was successful. The latter display the greatest E-module of 3.4 MPa in shear strength. All patches exhibited low cytotoxicity and can therefore find potential use in future treatments of bone fractures.

KEYWORDS: bone • fracture • fibre • reinforced • adhesive • FRAP • thiol-ene • primer • DOPA

INTRODUCTION

For most bone fractures treatments, it is desirable to mechanically support the fractured bone during natural bone repair to prevent pain, prolonged repair, and bone malformations. Conventional implant fixation often relies on the use of screw-fixated plates, applied by open surgery under general anaesthesia. Although this technique is often found effective, it also possesses some limitations. Because plates require drilling and screwing, a certain amount of strong and healthy bone is needed around the fracture. In cases where the bone is thin, weak, fragmented or close to sensitive tissues, fixation can therefore be difficult. Moreover, because screw anchored plates are applied by open surgery; the accessibility is somewhat limited and excludes the treatment of fractures in difficult locations.

As a result, an increased focus on developing minimally invasive techniques has been reported during the last years where cross-linked bone adhesives have shown to be promising candidates (1, 2). Because adhesives do not require drilling and can be distributed via minimal invasive surgery, their use for fracture stabilization is foreseen as ideal, especially for bone fragments in trauma surgery; fractures on thin, complex shaped bones; and fractures close to sensitive tissues. Cyanoacrylates, alkylene bis(oligolactoyl)-methacrylate, and a number of dental and fibrin-based adhesives are all promising bone adhesives that still fail because of poor mechanical properties or doubtful biocompatibility (3–6). One way to circumvent the problem of poor biocompatibility is to avoid acrylic polymer systems and investigate known nontoxic crosslinked systems such as thiol-ene adhesives. The radical initiated reaction between thiols and unsaturated double bonds (enes) yielding thio-ethers at benign conditions has been known since the 1920's. In fact, thiol-ene coupling (TEC) reactions emulate cysteine-based biological reactions and yield the bio-friendly thio-ether linkage (7). Indeed, this cross-linking strategy is highly desirable in a surgical environment, especially via UV curing, as it can be performed via minimally invasive optical fibres and with excellent tolerance to oxygen. Furthermore, the UV initiating strategy is harmless to the surrounding tissue in comparison to the thermal curing approach.

Although adhesive application of bone fractures is a promising method, it also introduces some challenges. Regardless of its chemical composition, the adhesive applied on the fracture surface is believed to interfere with natural bone repair and give rise to small bonding areas (8). As an alternative, fiber-reinforced adhesive patch (FRAP) fixation of bone fractures is believed to be superior to adhesive applied directly at the fracture ends (9). The shear strength of the FRAP can be tuned by the chemical nature of cross-linked components, type of fiber, and the number of fiber sheats included in the patch. Additionally, the interface between the cross-linked adhesive and bone surface needs to possess excellent bonding ability for optimal bearing properties. An inspiring adhesive mechanism found in nature is the mussels', *Mussels Byssus*, unique ability to strongly bond to various substrates, both in aqueous and dry environments. These extraordinary adhesive properties were identified a couple of decades ago and assigned to the

* Corresponding author. Tel: +46 8 790 4847 (H.V.H.); +46 8 7908748 (M.M.).
E-mail: hvh@kth.se (H.V.H.); malkoch@kth.se (M.M.).
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† School of Technology and Health, Royal Institute of Technology.
‡ Division of Coating Technology, Fibre and Polymer Technology, Royal Institute of Technology.
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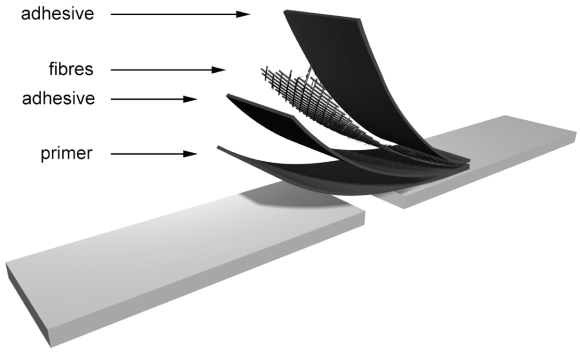


FIGURE 1. FRAP fabrication on bone.

mussel foot proteins (mfps), especially the sequences expressing the phenolic 3,4-dihydroxyphenyl-L-alanine (DOPA) residue (10−12). Depending on the surrounding pH, DOPA can be found in two forms: DOPA quinone and as a catechol (13). Although the quinone adduct plays an important role for cross-linking and solidification of DOPA, the catechol derivative give rise to hydrogen bonding, metal ligand complex formation and adhesion to inorganic surfaces. An example is the promising adhesion to hydroxyapatite (14−16), which is a key component in bone. DOPA's adhesion properties are highly desirable for various film applications; however, the nontoxic nature of the compounds is perhaps of most interest for in vivo purposes, especially their use as interfacial adhesive primers for bone fracture treatments.

Even though some papers report on the adhesion properties of phenolic DOPA components, no examples can be found in literature, to the best of our knowledge, exploring DOPA as primers for bone fractures treatments. Consequently, we herein investigate the role of different phenolic structures as primers for the fabrication of fibre reinforced thiol-ene patches for bovine femur bone fracture fixations. Catechol and quinone form of dopamine are investigated as a derived structure from DOPA aminoacid. Moreover, poly(parahydroxystyrene), p(PHS), is explored as a polymeric primer mimicking tyrosine and DOPA residues in the polyphenolic protein.

RESULTS AND DISCUSSION

To demonstrate the novelty of the proposed FRAP strategy for bone fixation applications, the commercial cyanoacrylate, Histoacryl, was initially investigated as a model system. FRAP fixations were manufactured by applying a thin layer adhesive followed by several layers of E-glass fibres and a finally top coat of adhesive, Figure 1. Six layers of fiber sheets were used throughout the study for direct comparison between the different FRAPs. Histoacryl was found to adhere well to wet bone and its use as a matrix for the embedment fibres exhibited excellent shear strengths of 3.8 MPa, patch 5, Table 1 and Figure 2. However, Histoacryl showed to be more suitable as a thin film adhesive than a matrix for fiber embedment. With increasing number of fiber layers, the curing of Histoacryl became insufficient and resulted in a brittle composite with signs of poorly cured compartments. Furthermore, the degradation mechanism of the cured Histoacryl matrix results in the formation of

Table 1. Patch Composition

patch	primer	matrix	conformation	shear strength (MPa)
1		thiol-ene		0.4
2	dopamine	thiol-ene	catechol	1.1
3	dopamine	thiol-ene	quinone	1.5
4	p(PHS)	thiol-ene		3.4
5		Histoacryl		3.8

formaldehyde adducts, which have been reported in numerous articles to induce infections and tissue necrosis and inhibit bone healing. This concern was supported in this study where a fully cured thin film of Histoacryl resulted in a 75−80% MG63 osteoblast cell survival after 72 h. These results further strengthen the cytotoxic nature of cyanoacrylates, and although the Histoacryl is approved for medical use, its employment as an in vivo adhesive for FRAP bone fixation seems less promising.

As a result, we investigated the TEC reaction between thiols and alkenes for the fabrication of a matrix that fulfil the requirements for a versatile FRAP protocol. From a surgical point of view, the thiol and the unsaturated monomers need to be miscible with processable viscosity, allowing efficient wetting of the fibres prior to cross-linking. Taking into account the large availability of commercial thiol and vinylic building blocks, we initially fabricated and examined polyethylene glycol diacrylate (PEG)-based FRAP, considering the excellent hydrophilicity of the starting components. Unfortunately, PEG-based FRAPs were found to exhibit poor adhesion to bone surfaces as well as low E-modules. Subsequently, we capitalized on the use of commercially available triazine building blocks, i.e., the trivalent tris[2-(3-mercaptopropionyloxy)ethyl] isocyanurate **1** and 1,3,5-triallyl-1,3,5-triazine-2,4,6-trione **2**. The triazine building blocks were chosen as potential candidates based on the desirable E-modules reported by Hagberg et. al. (17). The two monomers displayed full miscibility without the use of any solvents. Their versatility towards UV cross-linking was monitored by Raman spectroscopy until quantitative disappearance of the thiols and allylic peaks, at 2580 and 1650 cm^{-1}, respectively. Interestingly, at an equimolar ratio, the monomers were efficiently cured after 2 seconds of irradiation at 1.66 J/cm^2, resulting in matrix **3**, Scheme 1.

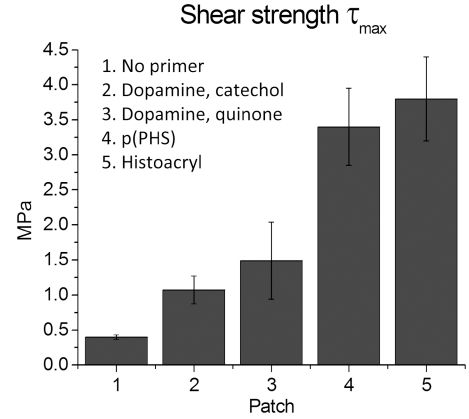
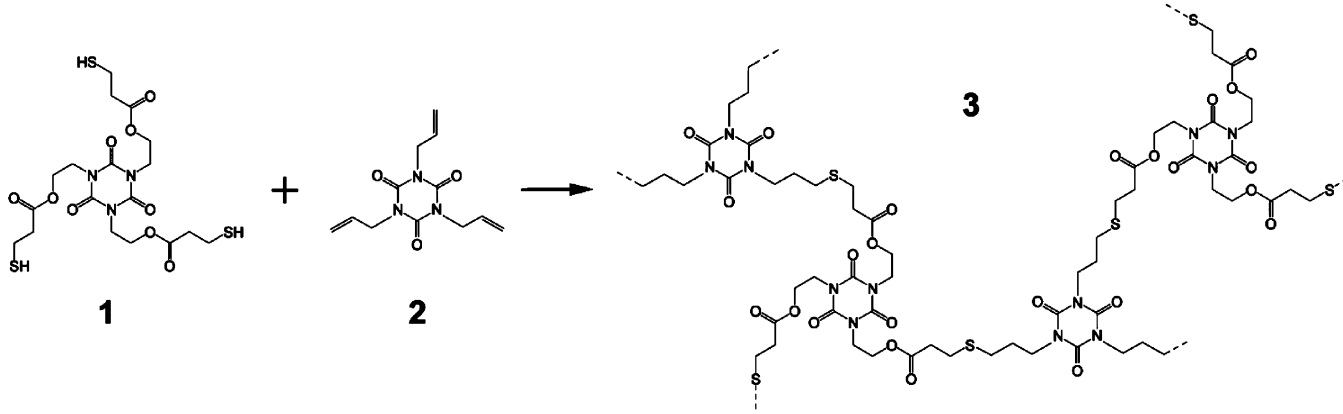


FIGURE 2. Maximum shear strength of FRAP bonded specimen, six replicas, using different primers.

Scheme 1. UV Curing for the Fabrication of a Thiol-ene Matrix



On the basis of the promising initial curing results and to examine the bone adhesion properties of the triazine matrix, we cured six sheets of E-glass fibres imbedded between two thiol-ene layers on bone without the use of any primer as adhesion enhancer, FRAP 1. The monomers were successfully cured on moist bone and the obtained FRAP excluded any detectable defects or uncured compartments, patch 1 and Table 1. Cytotoxic study on the triazine matrix revealed excellent nontoxic results with an MG63 osteoblast cell survival of 95−98 %. Unfortunately, the hydrophobic property of the thiol-ene matrix resulted in poor adhesion to moist bone, with low shear strength of 0.4 MPa, Figure 2.

To elevate the poor adhesion of the triazine-supported FRAP to moist bone surface, we introduced dopamine **4** as a hydrogen-bonding intermediate adhesive primer, Figure 3. A 2.5 wt % solution of dopamine **4** was prepared in an EtOH/H_2O mixture (90:10) and applied to wet bone tissue. Two FRAPs were fabricated to examine the influence of solvents on the adhesive properties. One was produced on a wet primer-bone surface and directly after applying the primer solution. The second FRAP was constructed on a moist primer-bone surface obtained after 1 min of evaporation prior to curing. From the two systems, the moist thin film primer displayed greatest FRAP adhesion, Figure 4, whereas the same system on wet bone demonstrated poor adhesion. This could be reasoned to the hydrophobic nature of the starting triazine components exhibiting poor adhering features toward wet bone surface.

The shear strength of FRAP, patch 2, on moist bone exhibited almost 3 times greater value of 1.1 MPa compared to patch 1. Furthermore, higher concentration of dopamine had no influence on the adhesion strengths of the fabricated FRAP. As such, all FRAP were produced on post-evaporated primer films containing 2.5 wt % dopamine **4**. To investigate the quinone form of DOPA, we oxidized dopamine **4** with NaOH at pH 8.5. Dopamine quinone was further used as a primer utilizing the solvent evaporation strategy and the fabricated patch 3 showed an increase in shear strength of 1.5 MPa compared to the catechol form, patch 2. As can be noted, the phenolic DOPA primers enhance the adhesion strength in the studied systems. The adhesion increase going from the catechol to the oxidized quinone form could possibly be due to the crosslinking via aryl−aryl coupling (di-dopamine formation) or via Michael-type addition reactions with amine groups. It is also likely that the DOPA-quinone interacts directly with the bone surface.

In an alternative approach, 2.5 wt % solution of the polymeric p(PHS) **5** phenolic primer (10 K) was applied in the same fashion as for the DOPA primer. Interestingly, the produced triazine-based FRAP with the p(PHS) as an interfacial primer exhibited improved shear strength with a value of 3.4 MPa. This can be comparable with the model Histoacryl-based FRAP with 3.8 MPa in shear strength, patch 5.

The excellent adhesion of the polyvalent p(PHS) may perhaps be due to a combination of chain entanglements and strong secondary forces.

In addition to the good mechanical results, neither p(PHS) or dopamine were found to induce any cytotoxicity using MG63 osteoblast-like cells. However, during the cytotoxicity test of dopamine-based material in CGM, it was noted that cells detached from the 96-well plates. Consequently, the ELISA procedure became unreliable and an ocular qualitative grading of the MTT staining was exploited. DOPA groups cell adherent effect is well-documented and also commercially available as Cell-tac, BD Biosciencies. Nevertheless, DOPA's ability to cause cell detachment from a substrate in solution

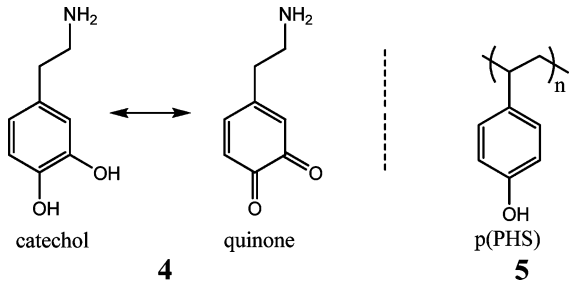


FIGURE 3. Chemical structures of (left) dopamine and (right) p(PHS) primers.

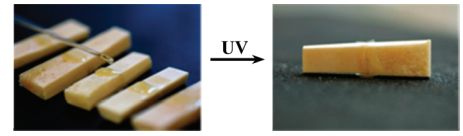


FIGURE 4. Facile FRAP strategy on moist bone substrates.

is somewhat interesting and should be further studied from a biocompatibility point of view.

Even though promising results were found for the developed FRAP, the key issue is if 3.4 MPa of shear strength is sufficient for safe bone fracture fixations. By using patch fixation instead of applying adhesive directly on the fracture, it is possible to tune the patch according to loading conditions on the fracture. Manipulating the number of fiber layers or increasing the adhesion area allows adjustments of the final strength of the patch. Additionally, a major consideration is the fracture location. The shear strength of 3.4 MPa implies a maximum shear load of 218 N for an 8 × 16 mm patch. This indicates the patch use for complex fractures in trauma surgery; fractures on thin, complex-shaped bones; and fractures close to sensitive tissues rather than highly loaded femur fractures. The FRAP fixation approach chosen in this study with patching outside the fracture is also believed to interfere less with the natural bone healing process than adhesives applied directly in the crevice of a fracture. The fractures used in this study were achieved by sawing the bones in two parts, which creates very fine and even fractures. In reality, most fractures are more rugged and complex in shape, and can in many cases add a certain amount of mechanical support to stabilized fractures.

CONCLUSIONS

In conclusion, triazine-based, fiber-reinforced adhesive patches were efficiently fabricated via the UV-induced thiol-ene chemistry for bone fracture stabilization purposes. To elevate the adhesion properties of FRAPs on fractured bone substrates, we discovered the phenolic dopamine and poly(parahydroxystyrene) as potential interfacial primers. In fact, the use of poly(parahydroxystyrene) as a primer generated bone-stabilizing FRAPs with shear strength values reaching that of the commercial Histoacryl. Additionally, the proposed thiol-ene patches demonstrated excellent cytocompatibility to MG-63 cells. Further studies are necessary to optimize adhesive chemistry and to fully investigate the properties of FRAP fixation. To improve adhesion, we are presently conducting an elaborative study on the bonding interfaces between bone to primer and primer to patch. Moreover, future investigation should include additional mechanical studies to complement tensile testing and sheer strength with other load scenarios, such as bending tests. For better understanding of load-bearing requirements, we have initiated a recent study using a finite element method to investigate the use of FRAP for stabilizing fractures in the cervical spine. Although DOPA-based FRAP exhibited low cytotoxicity, their cell detachment ability encourages further cytotoxicity investigations prior to animal model tests.

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Supporting Information Available: Materials, instrumentation, setup for cytotoxicity testm and patch formation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org

REFERENCES AND NOTES

(1) Gosain, A. K. *Plast. Reconstr. Surg.* **2002**, *109*, 2581–2583.
(2) Donkerwolcke, M.; Burny, F.; Muster, D. *Biomaterials* **1998**, *19*, 1461–1466.
(3) Giebel, G.; Rimpler, M. *Biomed. Tech.* **1981**, *26*, 35–40.
(4) Grossterlinden, L.; Janssen, A.; Schmitz, N.; Priemel, M.; Pogoda, P.; Amling, M.; et al. *Biomaterials* **2006**, *27*, 3379–3386.
(5) Camreron, J. L.; Woodward, S. C.; Pulaski, E. J.; Sleeman, H. K.; Brandes, G.; Kulkarni, R. K.; et al. *Surgery* **1965**, *58*, 424–430.
(6) Szep, S.; Kunkel, A.; Ronge, K.; Heidemann, D. *J. Biomed. Mater. Res., Part B* **2002**, *63*, 53–60.
(7) Hoyle, C. E.; Lee, T. Y.; Roper, T. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5301–5338.
(8) Woodward, S. C. *Ann. N.Y. Acad. Sci.* **1968**, *146*, 225.
(9) Nordberg, A.; Von Holst, H.; Brolin, K.; Beckman, A. *Biomed. Matr. Eng.* **2007**, *17*, 299–308.
(10) Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038–1040.
(11) Waite, J. H. *Int. J. Adhes.* **1987**, *7*, 9–14.
(12) Yu, M.; Hwang, J.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 5825–5826.
(13) Lee, H.; Dellatore, S. M.; Miller, M. M.; Messersmith, P. B. *Science* **2007**, *318*, 426–430.
(14) Chirdon, W. M.; O'Brien, W. J.; Robertson, R. E. *J. Biomed. Mater. Res., Part B* **2003**, *66B*, 532–538.
(15) Zhao, H.; Waite, J. H. *J. Biol. Chem.* **2006**, *281*, 26150–26158.
(16) Frank, B. P.; Belfort, G. *Biotechnol. Prog.* **2002**, *18*, 580–586.
(17) Hagberg, E. C.; Malkoch, M.; Ling, Y.; Hawker, C. J.; Carter, K. R. *Nano Lett.* **2007**, *7*, 233–237.

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