

# Photochemical tissue bonding using monomeric 4-amino-1,8-naphthalimides

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**Abstract.** Certain substituted naphthalimides have been shown to produce, on photochemical activation, mechanically viable bonds between a variety of tissue surfaces. It is believed that these compounds act as photochemically activated oxidants, catalyzing the formation of reactive intermediates in the extracellular matrices of approximated tissue surfaces. The condensation of these intermediates results in the formation of crosslinks between the intimate surfaces. Of particular interest is the application of this technique to the repair of tears in the typically unrepairable avascular zone of menisci. The menisci are collagen-rich fibrocartilaginous tissues that support up to 90% of the load across the knee joint and participate in important functions including shock absorption, joint stabilization, hyperextension prevention, and lubrication of the knee. Preliminary *ex vivo* and *in vivo* work in our laboratories has demonstrated that photochemically activated naphthalimides have significant potential for the repair of meniscal lesions. We describe preliminary *ex vivo* studies assessing the relative abilities of a variety of water-soluble monomeric 4-amino-1,8-naphthalimides to bond bovine knee meniscal tissue on visible light irradiation. © 2004 Society of Photo-Optical Instrumentation Engineers.  
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## 1 Introduction

Collagen is the predominant stress-bearing protein of connective tissues. Covalent crosslinks and hydrophobic interactions among collagen molecules serve to strengthen the fibrillar form of collagen.<sup>1–3</sup> When the organization of this material is disrupted due to trauma, lytic disease, or surgery, new collagen is formed at the site of injury.<sup>4–6</sup> This is followed by maturation and crosslinking of the collagen molecules. Proper healing is dependent on the correct reformation of the intra- and intermolecular interactions that strengthen the collagen fibrils. Traditional medical intervention in wound repair has relied on sutures and other sturdy mechanical devices to maintain tissues in apposition for a specific duration. However, there are numerous medical situations that are not amenable to these time-tested techniques. Accordingly, significant research effort has been focused on the development of adhesives for various medical applications.<sup>7</sup>

Tissue adhesives have been defined as substances that can form a polymeric material, where “polymerization must either hold tissues together or serve as a barrier to leakage.”<sup>7</sup> In line with this definition, most research and development in this area has been focused on technologies such as fibrin sealants,<sup>8,9</sup> cyanoacrylates,<sup>10</sup> hydrogels,<sup>11</sup> and protein/aldehyde composites.<sup>12</sup> Another approach to tissue bonding, often called laser tissue welding, involves the thermal coagulation and/or dehydration of tissue proteins themselves via the

application of laser energy.<sup>13,14</sup> The addition of light-absorbing dyes has been shown to improve heat transfer to the repair site during tissue welding.<sup>15</sup> A modification of this approach adds exogenous human proteins to the site of welding to act as a type of “protein solder.”<sup>16,17</sup> While each of these methods has certain advantages, all exhibit one or more shortcomings such as weak bond strength, excessive collateral tissue damage, and incomplete defect repair. Some have also been shown to interfere with tissue fluid diffusion or to initiate a localized inflammatory response. It remains a considerable challenge to develop bonding agents that provide strong bonds and yet do not act as physical or chemical barriers to natural wound healing.

Recognition that certain compounds efficiently crosslink soluble proteins on photochemical activation led to the realization that these compounds might also have the potential to photochemically bond tissues, especially those primarily composed of collagen (i.e., skin, cornea, and meniscus). In fact, this novel approach to tissue bonding is effective, and the application of the brominated bisnaphthalimide 1 and the hydrophilic bisnaphthalimide 2 (Fig. 1) to the *ex vivo* photoinduced crosslinking of cartilage, skin, and pure collagen, as well as *in vivo* repair of cartilage, has been reported.<sup>18–23</sup> The concept of forming a tissue bond by simply catalyzing the formation of covalent bonds between the tissue surfaces is innovative, and potentially solves the “barrier” problem inherent with traditional adhesives, as no foreign material is necessarily retained within the repair.

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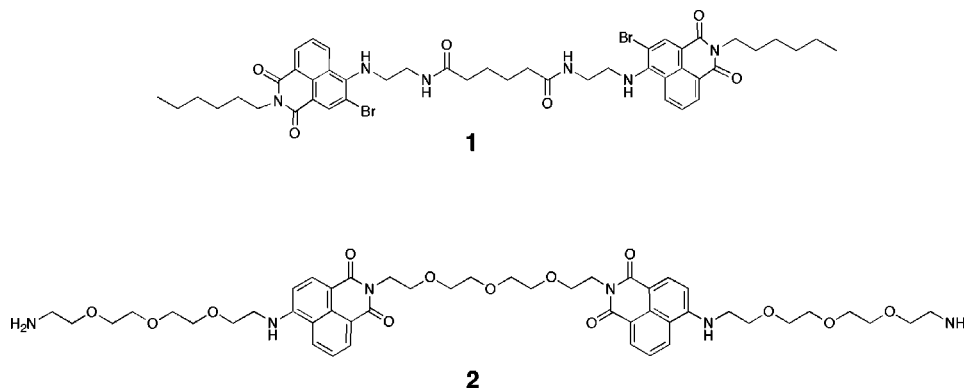


Fig. 1 Photoactive tissue-bonding naphthalimides.

To further explore this technology, to develop an understanding of the relationship between naphthalimide structure and bonding ability, and to hopefully develop improved photochemical tissue-bonding agents, we designed and synthesized a set of substituted 4-amino naphthalimides (Fig. 2). These naphthalimides absorb visible light ( $\lambda_{\max} \sim 430$  nm) and efficiently crosslink soluble protein.<sup>24</sup> Results of *ex vivo* experiments evaluating photochemical tissue bonding of bovine meniscus using these compounds are presented here.

## 2 Materials and Methods

### 2.1 Photoactive 1,8-Naphthalimides

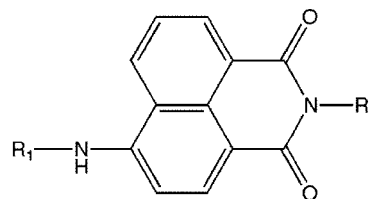
Naphthalimides 3 to 11 were synthesized and isolated as the water soluble trifluoroacetate (TFA) salts in a manner similar to that previously described.<sup>24,25</sup> Stock solutions (24 mM) were prepared in phosphate buffered saline, pH 7.4 (PBS). Additional PBS solutions of compound 8 were prepared in concentrations of 16, 18, and 27.75 mM.

### 2.2 Laser Delivery System

A Melles Griot diode laser with an emission of 457 nm was used. A 4-cm-focal-length biconvex lens expanded the laser beam, and a 1-cm-diam region located at the center of the expanding beam was defined by an iris diaphragm for illumination of the tissue to be bonded. The average power density within the 0.4-cm-diam spot was set at 2.7 W/cm<sup>2</sup>. The laser output power was routinely verified using a Coherent model 210 laser power meter.

### 2.3 Sample Preparation and Irradiation

Fresh bovine knee meniscus was obtained from a local slaughterhouse within a few hours of death. Meniscal specimens were dissected from the knee and sliced into 10×5×0.3 mm strips with a specially designed microtome apparatus. Each strip was further divided lengthwise into two strips, which were placed on top of a thin piece of polyurethane film on a glass slide. The two opposing surfaces of the tissue strips were each coated using approximately 30  $\mu$ L of PBS (control) or PBS naphthalimide solutions [Fig. 3(a)]. The coated surfaces were pressed together wrapped in the sheet of polyurethane film. An additional glass slide was then added to cover the sample, and two spring clips were employed to hold the



| Compound | R <sub>1</sub>  | R <sub>2</sub>   |
|----------|---|--|
| 3        | H <sub>3</sub> CO—CH <sub>2</sub> —CH <sub>2</sub> —                                  | —(CH <sub>2</sub> ) <sub>3</sub> —NH <sub>2</sub>                                  |
| 4        | H <sub>2</sub> N—(CH <sub>2</sub> ) <sub>2</sub> —(CH <sub>2</sub> ) <sub>2</sub> —   | —(CH <sub>2</sub> ) <sub>2</sub> —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> |
| 5        | HO—CH <sub>2</sub> —CH <sub>2</sub> —   | —(CH <sub>2</sub> ) <sub>3</sub> —NH <sub>2</sub>                                  |
| 6        | H <sub>2</sub> N—CH <sub>2</sub> —CH <sub>2</sub> —                                   | —(CH <sub>2</sub> ) <sub>2</sub> —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> |
| 7        | —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> —CH <sub>2</sub> —CH <sub>2</sub> — | —(CH <sub>2</sub> ) <sub>2</sub> —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> |
| 8        | H—  | —(CH <sub>2</sub> ) <sub>2</sub> —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> |
| 9        | H—  | —(CH <sub>2</sub> ) <sub>2</sub> —NH <sub>2</sub>                                  |
| 10       | CH <sub>3</sub> CH <sub>2</sub> —   | —(CH <sub>2</sub> ) <sub>2</sub> —N(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub> |
| 11       | H—  | —(CH <sub>2</sub> ) <sub>2</sub> —(CH <sub>2</sub> ) <sub>2</sub> —NH <sub>2</sub> |

Fig. 2 Naphthalimides used in this study.

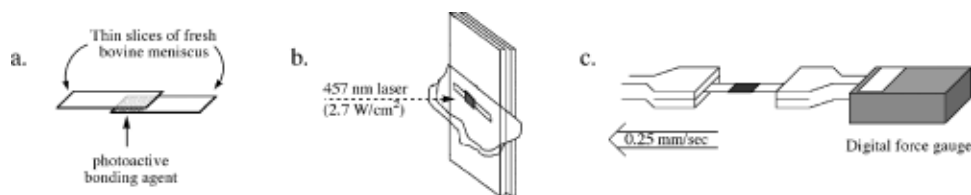


Fig. 3 *In vitro* bonding assay.

two slides together, providing a pressure of approximately  $3 \text{ kg/cm}^2$ . The specimens were then irradiated for 6 min at room temperature [Fig. 3(b)].

#### 2.4 Tensile Strength Analysis

After irradiation, the tissue samples were unwrapped from the polyurethane film and immediately connected to a microtensile testing system, composed of a variable speed motor with a worm gear and an Imada digital force gauge, for bond strength analysis [Fig. 3(c)]. The system was set at a strain rate of  $0.25 \text{ mm/s}$ . Bond strength was calculated as the ratio of tensile strength at failure to the overlapping bonded region (in kilograms per square centimeter), as measured by micrometer. Three to five replicate runs were performed for each compound. Results for the comparison between compounds were normalized to enable the use of data from two sets of experiments with slightly different total irradiance.

### 3 Results

The relative strengths of bonds formed in meniscal tissue using the nine photoactive naphthalimides are shown in Fig. 4. The bond strengths for these compounds (under the conditions of these tests) ranged from  $0.47 \text{ kg/cm}^2$  (for compound 3) to  $1.38 \text{ kg/cm}^2$  (for compound 11), with an average background bond strength of  $0.29 \text{ kg/cm}^2$  for control samples (irradiation/PBS with no naphthalimide). In every case, the failure occurred cleanly at the interface between the strips of meniscal tissue. Although these bonds are much weaker than a simple mattress suture ( $10.2 \text{ kg/cm}^2$  in human meniscus<sup>26</sup>), they are comparable to those previously reported for naphthalimide-mediated photochemical bonding of cartilage<sup>18,22</sup> and skin<sup>21</sup> and also to bonds formed in porcine aorta ( $\sim 1.1 \text{ kg/cm}^2$ ) using an albumin/indocyanine green thermal welding

technique.<sup>27</sup> A recent study<sup>28</sup> of the photosensitized adhesion of porcine skin using rose bengal reported much weaker bonding ( $0.06$  to  $0.15 \text{ kg/cm}^2$ ).

To examine whether this bonding technology exhibits a dose/response relationship, we also examined the bonding abilities of compound 9 at four different concentrations (Fig. 5). Although this limited experimentation did not reveal a clear linear correlation between bond strength and concentration, the bonding strength appears to plateau around approximately  $24 \text{ mM}$  (similar results were seen for other compounds; data not shown).

### 4 Discussion

Unfortunately the photochemical tissue-bonding results presented here are associated with large standard deviations, which seem to be inherent in this particular assay. The extreme variability observed in the available meniscal tissue is certainly a significant contributor to this poor reproducibility. This includes not only variation between samples derived from different animals or legs, but even within a single meniscus. Even so, a gross trend in the structure-activity relationship of this set of compounds can still be recognized. Compounds 3 to 6 are all poor reagents for adhering meniscal tissue. Those particular compounds contain either a nitrogen or an oxygen residue attached to the the 4' amino group by a two-carbon linker. On the other hand, compounds 8 to 11, which have either a hydrogen or a simple alkyl group attached to the 4' amine, are superior photochemical tissue bonding agents. One possible rationale for this behavior may come from recent research that has demonstrated the efficient formation of charge-separated species from 4-aminonaphthalimides,<sup>29</sup> which can readily extract electrons from a beta-amino substituted 4-amino substituents, resulting in fluorescence quenching. Accordingly, the differing activities of naphthalimides in the *ex vivo* tissue-bonding assay

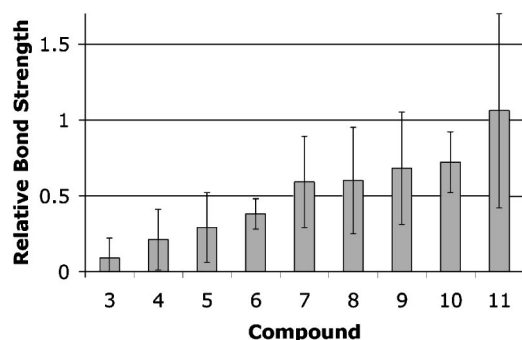


Fig. 4 Relative bond strengths.

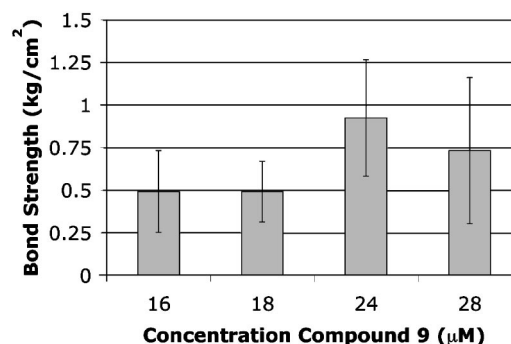


Fig. 5 Bond strength as a function of Naphthalimide concentration.

might be predicted by examining the potential for this non-productive intramolecular electron transfer. Additionally, as compounds 8 to 11 are also the most hydrophobic compounds tested [as determined by reverse-phase high-performance liquid chromatography (HPLC) retention], it is reasonable to propose that their enhanced tissue-bonding ability may reflect enhanced tissue or protein interactions related to their hydrophobicity. We continue to pursue mechanistic studies in this area, including studies aimed at explaining the role that the substituents on the 4-amino group play in tissue bonding.

Overall, the research described here has identified structural features that might be used to design new and improved photoactivated tissue-bonding agents. Further studies are focused on better identifying the molecular basis for this tissue-bonding technology, and in parallel, the development of more active naphthalimides for meniscal repair.

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