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Gelatin–alginate novel tissue adhesives and their formulation–strength effects

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ABSTRACT

Interest in tissue adhesives as alternatives for conventional wound-closing applications such as sutures and staples has increased in the last few decades due to numerous possible advantages, including less discomfort and lower cost. Novel tissue adhesives based on gelatin, with alginate as a polymeric additive and crosslinked by carbodiimide, were recently developed by our research group. The effects of the formulation parameters on the adhesives' function were investigated in the current study. We examined the effects of gelatin and alginate concentrations and their viscosities on the ability of the bioadhesives to bind soft tissues. The effect of the crosslinking agent's concentration was studied as well. A qualitative model describing these effects in terms of adherence mechanisms was developed. Our results show that the adherence properties of our new bioadhesives are achieved by a combination of two main mechanisms: mechanical interlocking and chemical adsorption. The former mechanism is probably more dominant. The polymer's molecular weight and concentration affect the mechanical interlocking through mobility and penetration ability, entanglement of the three-dimensional structure and crosslinking density. The crosslinking agent's concentration as well as the polymer's concentration affect the crosslinking density and contribute to higher strength, achieved through both the mechanical interlocking and the chemical adsorption mechanisms. Understanding the effects of the adhesives' components and their viscosities on the bonding strength enabled us to elucidate the bonding strength mechanisms. This can lead to proper selection of the adhesive formulation and may enable tailoring the bioadhesives to the desired applications.

1. Introduction

Lacerations and traumatic wounds are considered to be among the most prevalent scenarios encountered in hospitals and emergency rooms [1]. Reattachment of the lacerated soft tissue edges is traditionally performed using sutures or staples. Use of tissue adhesives, i.e. substances that have the ability to firmly attach lacerated tissues back together, as an alternative to these conventional applications has raised interest in the last few decades due to several major benefits. Tissue adhesives can be applied more quickly, may require less equipment and require a relatively less time-consuming procedure. Use of tissue adhesives prevents the painful procedure that is involved when using sharp instruments and was proven to be less expensive, without compromising the cosmetic outcome [2–4].

Although extensive efforts were made in the past, an ideal tissue adhesive has not been developed to date, probably due to the various rigid requirements that a substance must fulfill in order to serve as a medical tissue adhesive for clinical use [5–7]. Nonetheless, a few soft tissue adhesive products were approved for medical use – cyanoacrylates, fibrin and gelatin-based adhesives. These products were approved for restricted use only, due to the low biocompatibility of cyanoacrylates, which were crosslinked with formaldehyde or glutaraldehyde, or due to the low mechanical strength of the fibrin adhesives [8,9].

Although adhesives are currently used for various general applications, the principal aspects of adhesive bond formation have not been fully elucidated. Four main adhesion mechanisms were suggested [10,11]. The main two adhesion theories are:

(1) Mechanical interlocking – adhesion as a result of penetration of the adhesive into porosities or irregularities on the surface of the adherends.
(2) Chemical adsorption – adhesion as a result of the creation of intramolecular primary bonds (ionic, covalent, and metallic) or intermolecular secondary bonds (such as van der Waals and hydrogen bonds) between the adhesive agent and molecules on the surface of the adherends.

For most systems, the adhesion mechanism is considered to be a combination of mechanical interlocking and chemical adsorption.

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The proportionate contribution of each of the two adhesion mechanisms to the final bonding strength is variable and is affected by the adhesive system type, the surface roughness and the environment.

Gelatin, a water-soluble natural polymer derived from collagen, has become one of the most investigated materials for tissue adhesives due to its suitable natural properties. Gelatin is considered as biocompatible, biodegradable and nonimmunogenic [12]. It can form physically crosslinked hydrogel structures [13], has a natural tacky behavior in solution and is highly accessible in nature [14]. In spite of its promising qualities, the mechanical strength of physically crosslinked gelatin adhesives is not sufficient as an adhering substance on its own [14]. A chemical crosslinking agent and a polymeric additive (with suitable available functional groups for the crosslinking reaction) were therefore usually added to the solution in a wide range of published attempts, in order to create gelatin-based hydrogel formulations with suitable mechanical properties for soft tissue adhesion [6,14–17].

Novel tissue adhesives based on a combination of gelatin with an alginate polymeric additive and crosslinked by carbodiimide have recently been developed and studied by us [18,19]. Carbodiimide, which is mainly used for modification and conjunction of proteins and other biological macrostructures, was chosen as the crosslinking agent since carbodiimides and their crosslinking byproducts have been reported to be less cytotoxic than other conventional crosslinking agents such as formaldehyde and glutaraldehyde [20]. We tested the cytotoxicity effect of the bioadhesives on fibroblast cells and found that at relatively low EDC concentrations (less than 15 mg ml^{-1}), the cell viability is high (89–100%), while relatively high EDC concentrations (15 and 20 mg ml^{-1}) resulted in a cell viability which is still higher than 70% [18].

Alginate is a natural polysaccharide which is extracted from marine algae and was chosen to be the polymeric additive for the gelatin adhesive in the current research. As well as being a natural viscosity modifier with bioadhesive nature, alginate is also a natural source for a high concentration of carboxylic groups which are essential for the crosslinking reaction of carbodiimides. The carbodiimide couples to a carboxylic group (originally from the gelatin or the alginate) to form an o-iso-acylurea derivative which is highly reactive and has an extremely short life. This activated structure goes through a nucleophilic attack by a primary amino group (originally from the gelatin) to form an amide bond. As a result of the nucleophilic attack, a urea molecule (derivative of the carbodiimide type) is released as a byproduct [21]. Since lacerated tissues contain exposed amino and carboxylic groups which can take part in the crosslinking reaction, our adhesive has the potential to be especially attractive for tissue adherence.

The effects of the formulation parameters on the adhesive's function were investigated in the current study. We examined the effects of gelatin and alginate concentrations and their viscosities on the ability of the bioadhesives to bind to soft tissues. The effect of the crosslinking agent's concentration was studied as well. A qualitative model describing these effects in terms of adherence mechanisms is presented.

2. Materials and methods

2.1. Materials

Three types of gelatin "type A" from porcine skin with different Bloom numbers (90–100, 175 and 300), alginic acid sodium salts with low (LV) and high viscosity (HV) of 136 cP (0.136 Pa s) and 2690 cP (2.69 Pa s), 2% (25 °C), respectively, and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich, Rehovot, Israel.

2.2. Adhesive preparation

Adhesive preparation was based on dissolving various amounts of gelatin and alginate (Ge–Al) powders in distilled water, under heating up to 60 °C. The crosslinking agent (EDC) was added to the Ge–Al solution prior to the adhesive's use. All studied formulation series are presented in Tables 1–3. The gelatin–alginate–EDC formulations and their component concentrations are presented in the form of Ge–*X*:Al–*Y*:EDC–*Z*, where *X*, *Y* and *Z* are the concentrations of gelatin (Ge), alginate (Al) and EDC in mg ml^{-1}, respectively. Sometimes only the Ge–*X*:Al–*Y* combination is presented, and the EDC concentration is indicated separately. The pH values of the studied solutions are ~6.

2.3. In vitro bonding strength measurements

Porcine skin (Kibbutz Lahav, Israel) was used as a soft tissue model for investigating the adhesive's parameters and their effects

Table 1
The studied adhesive formulations with different EDC concentrations and various gelatin–alginate combinations.

Gelatin (90–110 Bloom) concentration (mg ml^{-1})	LV alginate concentration (mg ml^{-1})	EDC concentration (mg ml^{-1})
200	40	5
		10
		15
		20
300	30	5
		10
		15
		20

Table 2
The studied adhesive formulations with different gelatin Bloom numbers.

Gelatin concentration (mg ml^{-1})	Gelatin Bloom number	LV alginate concentration (mg ml^{-1})	EDC concentration (mg ml^{-1})
200	90–110	40	20
	175		
	300		

Table 3
The studied adhesive formulations with different alginate concentrations and viscosities under various gelatin concentrations.

Gelatin (90–110 Bloom number) concentration (mg ml^{-1})	Alginate concentration (mg ml^{-1})	Alginate viscosity[a]	EDC concentration (mg ml^{-1})
200	10	LV	20
		HV	
	20	LV	
		HV	
	30	LV	
		HV	
	40	LV	
300	10	LV	
	20		
	30		
400	10		
	20		
	30		

[a] LV: low viscosity (0.136 Pa s); HV: high viscosity (2.69 Pa s).