Mucoadhesion and Bioadhesion:
Mechanisms, Experimental Techniques and Development of New Systems

Nicholas A. Peppas
Departments of Chemical and Biomedical Engineering, and Pharmaceutics
The University of Texas at Austin
Austin, Texas 78712, USA
Bioadhesion

- Need of Bioadhesive Controlled Release Systems
- Slow Absorption of Certain Drugs
- “Immobilization” on Desirable Sites (e.g., Intestine, Stomach)
Principle of Bioadhesion

Strong Interactions between Chemical Groups of the Polymer Carrier and the Mucus Lining of the Tissue may keep Controlled-Release Device in Contact with Tissue for Desirable Time.

Application Sites

Nasal Cavity, Buccal Cavity, Stomach, Intestine, Urinary Bladder, etc.
Mucoadhesion Advantages

- Immobilization of a drug to a desired organ
- Increased duration of contact
- Topical delivery allows drug absorption directly into tissues
- Prolonged residence time of drug in the body
- Prevention of first-past metabolism of drugs by liver
- Increased bioavailability
The Mucus

- Bioadhesion is Related to the Interaction of Polymers with Mucus

- The Mucous Layer is a Highly Viscous Product Secreted by the Goblet Cells of the Tissue which Coat the Epithelial Cell Surface.
Mucus Production

- Viscous secretion by goblet cells
- Forms a variable, uneven coating over epithelial cell surface
- Continuous or intermittent secretion process with approximate renewal rate of 6 hours
- Irritation or disease tends to result in hypersecretion and a faster renewal rate
- Attachment of a polymeric adhesive device decreases renewal rate
The Mucus Layer

- **Components:**
  - Glycoproteins (Mucins)
  - Proteins
  - Lipids
  - Inorganic Salts

- **Composition:**
  - Lipids and Glycoproteins (0.5 – 1%)
  - Electrolytes (1%)
  - Proteins (0.5 – 1.0%)
  - Water (95%)
Glycoprotein Network

- A Glycoprotein Network, Highly Swollen in Aqueous Electrolytic Solution is the Main Constituent of Mucus.

- Mucus Glycoproteins Consist of a Protein Core with Carbohydrate Side Chains Covalently Attached.
Glycoprotein Network

- Long Chain, Branched Oligosaccharides are Attached to the Peptide Backbone, Predominantly by O-Glycosidic Linkages via the Serine and Threonine Amino Acid Components.

- The Whole Structure is Held Together by Disulphide and Secondary Bonds.

- Molecular Weight of $2 \times 10^6$ with Subunits of $10^5$
Drug carrier

Lumen fluid

Mucous layer

Epithelial cell layer
Polymers Used as Mucoadhesives

* Sodium Carboxymethyl cellulose (NaCMC)
* Hydroxypropyl cellulose (HPC)
* Sodium Alginate
* Poly(acrylic acid) (PAA)
Mechanisms of Bioadhesion

- Interpenetration by Dangling Chain Ends
- Swelling of Polymer Matrix by Soft Tissue
- Interfacial Interaction of Functional Groups
- Electrostatic Interaction
Mechanisms of Bioadhesion

• Physical or mechanical bonds

• Secondary chemical bonds
  – hydrogen bonding between polymer and tissue groups
    » hydroxyl
    » carboxyl
    » sulfate
    » amino groups
  – Van der Waals forces

• Primary bonds
Chain Interpenetration and Adhesion

Polymer adhesion can be enhanced by *chain interpenetration*

Structural Requirements for Good Mucoadhesion

**Molecular Weight**

The Longer the Chain Length, the Better the Mucoadhesion (Up to a Certain Molecular Weight)

The Maximum Adhesion of Poly(acrylic acid) was Observed with Molecular Weight 750,000 (Carbopol 910)

Types of Functional Groups Contributing to Mucoadhesion

1. Charged Functional Groups (-OH, -COOH)
2. Hydrogen Bond-Forming Functional Groups
Common Mucoadhesive Polymers

Carbopol 934

1. High Molecular Weight Polymer of Acrylic Acid (Noveon Chemical Co.)

Hydroxypropyl Cellulose and Carboxymethyl Cellulose

2. A Mixture of Carbopol and Hydroxypropyl Cellulose


3. Combinations with CMC, Pectin and Gelatin

4. Gums (Alginates, Carrageenan, Gum Arabic, Pectin)
   Usually Form a Mucilagenous Solution in Water and Become Sticky
Mucoadhesive Dosage Forms

Oral

Crosslinked Poly(acrylic Acid) Effectiveness

Buccal

Carbopol 934 and Hydroxypropyl Cellulose

Ocular

Some early studies (1993) showed increased Bioavailability of Progesterone in systems with Polycarbophil

Nasal

Carbopol 934 has been used in studies of Delivery of Insulin
Successful Buccal Bioadhesive Systems

(T. Nagai, J. Controlled Release, 2, 121 (1985))


Assigned to Teijin Co., Tokyo
Successful Buccal Bioadhesive Systems

- They Prepared Adhesive Dosage Forms Containing Hydroxypropyl Cellulose and Carbopol® 934

- Triamcinolone Acetonide for Treatment of Buccal Aphthae: This System is Commercially Available in Japan Under the Name Aftach® (Teijin Co.)
Classification of Bioadhesive Polymers


- They Presented Efforts Towards Development of Oral Bioadhesive Systems (8-12 Hours Adhesion)

- Using a Fluorescence Spectroscopic Technique they Classified the Following Polymers from Best to Worst Adhesive Strength

- Good Bioadhesive Polymers: CMC, Hyaluronic Acid, Poly(acrylic acid)
## TRANSMUCOSAL DELIVERY USING BIOADHESIVE SYSTEMS

<table>
<thead>
<tr>
<th>Product</th>
<th>Bioadhesive agent</th>
<th>Pharmaceutical form</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccastem®</td>
<td>Polyvinylpyrrolidone (PVP), Xanthan gum and locust bean gum</td>
<td>Buccal tablet</td>
<td>Reckitt Benckiser</td>
</tr>
<tr>
<td>Corlan pellets®</td>
<td>Acacia gum</td>
<td>Oromucosal pellets</td>
<td>Celltech</td>
</tr>
<tr>
<td>Suscard® Gaviscon</td>
<td>Hydroxypropyl methylcellulose, Sodium alginate</td>
<td>Buccal tablet</td>
<td>Forest Reckitt Benckiser</td>
</tr>
<tr>
<td>Liquid®</td>
<td></td>
<td>Oral liquid</td>
<td>Reckitt Benckiser</td>
</tr>
<tr>
<td>Gynol-li®</td>
<td>Sodium carboxymethyl cellulose and PVP</td>
<td>Vaginal gel</td>
<td>Janssen-Cilag</td>
</tr>
<tr>
<td>Zidovudine®</td>
<td>Carbomer</td>
<td>Vaginal gel</td>
<td>3M</td>
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<tr>
<td>Orabase®</td>
<td>Pectin, gelatin</td>
<td>Oral paste</td>
<td>ConvaTech</td>
</tr>
<tr>
<td>Corsodyl gel®</td>
<td>Hydroxypropyl methylcellulose</td>
<td>Oromucosal gel</td>
<td>GSK</td>
</tr>
<tr>
<td>Nyogel®</td>
<td>Carbomer and poly(vinyl alcohol)</td>
<td>Eye gel</td>
<td>Novartis</td>
</tr>
<tr>
<td>Pilogel®</td>
<td>Carbomer</td>
<td>Eye gel</td>
<td>Alcon</td>
</tr>
<tr>
<td>Timoptol-LA®</td>
<td>Gellan gum</td>
<td>Eye gel-forming solution</td>
<td>MSD</td>
</tr>
<tr>
<td>Aci-Jel®</td>
<td>Tragacanth, acacia</td>
<td>Vaginal gel</td>
<td>Janssen-Cilag</td>
</tr>
<tr>
<td>Crinone®</td>
<td>Carbomer</td>
<td>Vaginal gel</td>
<td>Serono</td>
</tr>
</tbody>
</table>
Tensiometric Technique

[G. Ponchel et al., J. Controlled Release, 5, 129 (1987)]

[N.A. Peppas et al., J. Controlled Release, 5, 143 (1987)]

[G. Ponchel et al., Intern. J. Pharm., 38, 65 (1987)]


A Polymeric Tablet Preswollen by Water (SP) and Kept in Contact with Mucus (M) has an Initial Length, \( l_0 \), as Shown in (a). It is Extended to a New Length, \( l_1 \), as in part (b) and the Stress Developed is Followed as a Function of Time.
Variation of the Force Necessary for Detachment of the Polymeric System from the Bovine Sublingual Mucus Surface at 26°C as a Function of Elongation. Experiment Carried out in a Drug-Free Tablet Containing 50 wt% PAA, with Water-Preswollen Surface (for 10 min).

Associated Work of Adhesion.
Falling Liquid Film Method

- Preparation of “Mucoadhesive” Particles
- Preparation of Intestinal Tissues
- Formation of Liquid Film on the Intestinal Segment
- Adhesion of Polymer-Coated Particles
- Collection of the Eluted Particles in Solution
- The Fraction of Particles Adsorbed on the Mucous Layer, $F_a$
Falling Liquid Film Method

\[ F_a = 1 - \frac{N}{N_0} \]

\( N_0 \) and \( N \) are the Particle Concentrations Entering the Intestinal Segment and Leaving the Segment, Respectively.

Schematic Diagram of the Falling Liquid Film Perfusion System
Mucoadhesive Capacity Increases with Adhesion Promoters

By adding PEG to PAA-based mucoadhesives, mucoadhesion is improved

Improvement of Mucoadhesin by Increasing Chain Interpenetration

- PAA
- Mucus Layer
- PEG
Mucoadhesion of PEG-containing Complexation Hydrogels

Displacement (cm)

Detachment Force (N)

pH = 3.2
pH = 7.4
Tethered Polymer Chains as Mucoadhesion Promoters


Tethered Polymer Chains

- Tethered polymer chains are polymer chains attached to a point, a line, a surface or an interface by their chain ends.
Free Chains as Mucoadhesion Promoters

- Free polymer chains are loaded into the synthetic gel.
- When two gels contact, free chains diffuse across interface, and form interfacial connectors.
- De Ascentiis et al. experimental work in 1994
Tethered Chains as Mucoadhesion Promoters

- Surface structures are controllable.
- Bulk structures may be optimized separately.
To design an experiment in order to measure the microscopic interaction between tethered polymers and a mucous layer in a physiologically relevant environment.
Mucoadhesive Polymers as Enzyme Inhibitors and Penetration Enhancers

Lehr has reported that PAA (notably Carbopol 934) was “able to inhibit the degradation of various peptides and proteins, such as insulin or hemoglobin by the proteolytic enzymes of homogenized rat intestinal mucosal cells.”


Promising data for insulin delivery with oral formulation? No such successful system has come up from this work.
Mucoadhesive Polymers as Enzyme Inhibitors and Penetration Enhancers

Promising data for insulin delivery with oral formulation? No such successful system has come up from this work.

But very successful new developments can be found in:


Fig. 3: Hypothetical mechanism of opening the tight junctions between mucosal epithelial cells upon contact with dry mucoadhesive polymers, such as for example starch microspheres. Top: the dry materials swells by attracting water from the mucus gel layer. Bottom: As a consequence of mucus dehydration, the cells shrink and intercellular junctions are widened, allowing the influx of water and macromolecules along the paracellular route.
Mathiowitz has reported new bioerodible microparticulate formulations based on polyanhydrides, which can be used for oral release of insulin.

[E. Mathiowitz et al., Nature, 386, 410 (1997)]

Mathiowitz showed that biologically adhesive “engineered polymer microspheres made of biologically erodible polymers, which displayed strong adhesive interactions with gastrointestinal mucus and cellular linings … could be developed as delivery systems to transfer biologically active molecules (dicumarol, insulin, plasmid DNA) to the circulation.”
Figure 4 Reaction to glucose load in rats fed 1.5% (w/w) insulin-loaded poly(FA:PLGA) PIN microspheres (green, N = 6), 20 IU soluble insulin in saline (black, N = 8), and saline buffer (red, N = 7). All data are mean ± s.e.
Oral Delivery of Proteins

- pH ~2
- Mucosa
- Epithelial Cells
- pH ~7
- Enzyme

Bloodstream

Diagram showing the oral delivery of proteins through the mucosa of epithelial cells at pH ~2 and the bloodstream at pH ~7.
Oral Delivery of Proteins

Challenges

- Protection of the drug from:
  - The acidic environment in the stomach
  - Degradation by proteolytic enzymes in the GI tract
- Penetration and absorption of drug across the intestinal mucosa and epithelium

Carrier Requirements for Oral Delivery

- Protect drug in the harsh environment of the GI tract
- Release drug at targeted site of absorption (upper small intestine)
- Increase residence time at absorption site
- Biocompatible

## Molecular Design of Drug Carrier

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protect drug in the harsh environment of the GI tract</td>
<td>Complexation hydrogels</td>
</tr>
<tr>
<td>Release drug at targeted site of absorption (upper small intestine)</td>
<td>Environmentally responsive hydrogels</td>
</tr>
<tr>
<td>Increase residence time at absorption site</td>
<td>Mucoadhesion and cellular adhesion</td>
</tr>
<tr>
<td>Biocompatible</td>
<td>Incorporate PEG chains</td>
</tr>
</tbody>
</table>
Interpolymer Complexation in P(MAA-g-EG) Hydrogels

- Complex formed due to hydrogen bonding between the protons on the carboxylic acid with the etheric groups of the grafted PEG chains.
- Forms in aqueous media within a narrow range of pH, ionic strength and solvent composition.
- At higher pH, COOH groups ionize, breaking the complex and leading to network swelling.
Physiologically Sensitive Hydrogels

PEGMA + TEGDMA + MAA

PMAA

PEG Crosslink

PEG
Molecular Design of the Drug Delivery System

Addition of PEG critical for:

- Complexation
- Mucoadhesion
- Drug stabilization

Mucoadhesion

Drug stabilization

Complexation

Mucosa
Network Mesh Size in P(MAA-g-EG) Hydrogels as a Function of pH and copolymer composition at 37° C

PEG MW = 1000
Dynamic Swelling Conclusions

- Both pH and ionic strength are factors in the swelling of P(MAA-g-EG) disks
  - Swelling begins at a pH = 5.5
  - Swelling ratio, q, was greatest at I = 0.1M
- Swelling at 5 minutes approximates what happens in the body

Particle diameter = 100µm
D is the diffusion coefficient of H₂O in dry polymer

\[ t = \frac{l^2}{D} = \frac{(0.01\text{cm})^2}{10^{-6}\text{ cm}^2/\text{s}} = 1\text{min } 40\text{sec} \]

- t = time
- l = length
- D = diffusion coefficient
Effect of Complexation on Drug Diffusion

<table>
<thead>
<tr>
<th>Composition</th>
<th>$D_{6.8}/D_{1.2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 MAA/EG</td>
<td>550</td>
</tr>
<tr>
<td>4:1 MAA/EG</td>
<td>66</td>
</tr>
<tr>
<td>PMAA</td>
<td>3</td>
</tr>
</tbody>
</table>


Mucoadhesion

Stomach

Decomplexation

Upper small Intestine

Mucosa
Increasing Residence Time: Incorporation of a Cellular Adhesion Peptide

- Arg-gly-asp-ser (RGDS) is a 4 sequence amino acid which promotes cell adhesion through an integrin receptor
- Functionalize PEG chain with RGDS
- Cell adhesion requirements:
  - Minimum spacer distance for adhesion (~35Å)
  - Minimum surface density of RGDS (1 fmol/cm²)
Tight Junction

Polymeric Carrier

Systemic Circulation

Protein

Proteolytic Enzymes

Mucosa
New Insulin Delivery System

Stomach

Upper Small Intestine

$\xi \sim 50 \text{ Å}$

$\xi \sim 200 \text{ Å}$

Crosslinked microparticles of poly(methacrylic acid) grafted with poly(ethylene glycol)
Blood Glucose Response in Healthy and Diabetic Wistar Rats

![Graph showing blood glucose levels over time for healthy and diabetic animals. The y-axis represents serum glucose (% of initial level) ranging from 0 to 140, and the x-axis represents time (h) ranging from 0 to 8. The graph compares healthy and diabetic animals, with a dashed line at 100%.](image)
In-Vivo Blood Glucose Response: Dose Effects

![Graph showing blood glucose response over time with different insulin doses.](image-url)
Serum Glucose in Healthy Rats Following Intestinal Administration

Serum Glucose and Insulin Levels in Healthy Rats Following Intestinal Administration

- **Solution - glucose level**
- **Polymer - glucose level**
- **Solution - insulin level**
- **Polymer - insulin level**

Time, t (h):
- 0
- 1
- 2
- 3
- 4

Serum Glucose (% Initial Level):
- 0
- 40
- 80
- 120
- 160

Serum Insulin (mU/ml):
- 0
- 25
- 50
- 75
- 100
- 125
- 150

10 IU/kg Doses
Experimental Design

**Drug**: Human insulin (10, 25, and 50 IU/kg)

**Preparations**: Insulin Loaded Polymer (ILP)
- MAA : EG = 1 : 1  \(L-ILP\) (200-300 \(\mu\)m)
- SS-ILP (1-50 \(\mu\)m)
- \(NS(1)-ILP\) (200-400 nm)
- AA : EG = 2 : 1  \(NS(2)-ILP\) (200-400 nm)

**Absorption study**: Male Wistar rats (180-200g)
*In vivo* absorption study

**Measurements**: Plasma insulin level (EIA)
- Blood glucose level (Glucose oxidase method)

Blood Glucose and Plasma Insulin Levels
Following Oral Administration of SS-ILP (1-50 μm)

**Glucose level**

![Graph showing blood glucose level over time for different groups, indicating significant differences and mean ± S.E. (n = 8-11).](image)

**Insulin level**

![Graph showing plasma insulin level over time for different groups, indicating significant differences and mean ± S.E. (n = 8-11).](image)

Each value represents mean ± S.E. (n = 8-11).

* p<0.05; ** p<0.01 compared with control.
## Comparison of Bioavailability

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Dose (IU/kg)</th>
<th>AUC (% glu. reduc. · h)</th>
<th>BA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>1.5 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>L-ILP</td>
<td>50</td>
<td>0.2 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>SS-ILP</td>
<td>10</td>
<td>52.5±12.5</td>
<td>19.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>115.9±56.7</td>
<td>18.1 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>51.5±10.3</td>
<td>11.8 ± 0.3</td>
</tr>
<tr>
<td>NS(1)-ILP</td>
<td>50</td>
<td>8.6 ± 4.2</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>NS(2)-ILP</td>
<td>50</td>
<td>23.6 ± 6.0</td>
<td>11.6 ± 0.4</td>
</tr>
</tbody>
</table>

BA denotes bioavailability compared to S.C. Each value represents mean ± S.E.
An unexpected discovery!

Significantly reduced proteolytic activity of enzymes on insulin in the presence of the novel carriers

A.C. Foss and N.A. Peppas,
“Investigation of the Cytotoxicity and Insulin Transport of Acrylic-based Copolymer Protein Delivery Systems in Contact with Caco-2 Cultures,”

J.E. López and N.A. Peppas,
“Cellular Evaluation of Insulin Transmucosal Delivery,”
The graph shows the percentage of bioactive insulin remaining over time for different treatments. The x-axis represents time in minutes (0 to 60), and the y-axis represents the percentage of bioactive insulin remaining (0% to 100%). The treatments compared are:

- **Insulin + polymer in PBS**
- **Insulin + polymer**
- **Insulin**

The graph indicates a significant decrease in bioactive insulin for all treatments over time, with the combination of insulin and polymer in PBS showing the least decrease compared to the other treatments.
Caco-2 Cells as GI Model

- **Advantages**
  - Spontaneously differentiate
  - Produce enzymes
  - Posses tight junctions
  - Develop microvillii
  - Transport of inorganic molecules correlates well with the in vivo absorption

- **Disadvantages**
  - Do not produce mucus
  - The properties are determined by the passage number
Caco-2 cell monolayers at confluency

Passage # 74
6 days after seeding
Magnification: X20
Cell Studies

- Cytoxicity
  - Determination of the cell viability after contact with the studied system.
- Transepithelial electrical resistance (TEER)
  - Measurement of the paracellular ion flux. This is an indication of the membrane integrity.

Costar® Transwell Insert-clear
Expanded View of Transwell Insert-clear

Polycarbonate filter with pore size of 0.4 or 3.0 µm
Effect of Microsphere Composition and Concentration on Caco-2 Cells

Dispersing medium: HBSS containing Ca-Mg
Each bar represents the normalized mean ± STDV (n=16)
Transepithelial Electrical Resistance

- Caco-2 cells were seeded on Costar® Transwell-Clear with a polycarbonate membrane (pore size: 0.4 µm) at a density of 52,500 cells/cm² and cultivated in DMEM at 37°C in an atmosphere of 5% CO₂ and 90% R.H. for 21 days.
- The cell monolayers grown on the Transwells were washed twice with HBSS and further incubated until a constant resistance value was obtained.
- The medium containing hydrogel microspheres (10 mg/mL) was pH equilibrated and sterilized.
- The suspension was placed in contact with the cell monolayer in the apical side.
Transepithelial Electrical Resistance

Apical Chamber

Monolayer

Filter

Basolateral Chamber

Microvilli

Tight Junction

Membrane Filter
EVOM® Volt-ohm Meter with a Chopstick-type Electrode
Measurement of TEER
Measurement of TEER
Measurement of TEER
Effect of Molar Ratio of MAA and EG Repeating Units in P(MAA-g-EG) Hydrogels on the TEER in Caco-2 Cell Monolayers at 37 °C

Dispersing media: Ca- and Mg-Free Hank's balanced salt solution (pH 7.4)
Number of EG repeating units of PEG graft chains: 23
Dose of each P(MAA-g-EG) powder was fixed at 10 mg/well
Each point represents the mean ± S.D. (n=3)
Insulin Transport

• Caco-2 cells were seeded on Coster® Transwell-Clear with a polycarbonate membrane (pore size: 3.0 µm) at a density of 52,500 cells/cm² and cultivated in DMEM at 37°C in an atmosphere of 5% CO₂ and 90% R.H. for 21 days

• The cell monolayers grown on the Transwell were washed twice with HBSS/CMF and equilibrated for 60 min

• The media were replaced by the HBSS/CMF containing insulin and gel particles at concentration to be 10 mg/each well

• At specific time points, 0.1 ml of sample was withdrawn from the basolateral side and same volume of fresh media was added

• The insulin concentration was determined by EIA-kits
Effect of P(MAA-g-EG) Hydrogel Application to the Mucosal Side on Time Course of Insulin Permeation across Caco-2 Cell Monolayers

Dose of hydrogels with 1:1 molar ratio of MAA/EG and 23 EG repeating units: 10 mg/well
Transport media: Ca- and Mg-free Hank’s balanced salt solution (pH 7.4)
Initial concentration of insulin, $C_o$, = 163 µg/ml (4.4 IU/ml)
Each point represents the mean ± S.D. (n=3)
## Companies developing oral delivery systems for proteins and peptides

<table>
<thead>
<tr>
<th>Company</th>
<th>Technology</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotech Australia (Roseville, NSW/variou...</td>
<td>Uses vitamin B12 as carrier for oral delivery: also uses nanoencapsulation technologies</td>
<td>LH-RH, G-CSF, erythropoietin</td>
</tr>
<tr>
<td>Cortecs (Isleworth, UK)</td>
<td>Oral delivery</td>
<td></td>
</tr>
<tr>
<td>DepoMed Inc. (Foster City, California)/J...</td>
<td>Oral drug delivery systems: Gastric Retention System and Reduced Irritation System</td>
<td>Oral salmon calcitonin, oral insulin</td>
</tr>
<tr>
<td>Eli Lilly &amp; Co. (Indianapolis)</td>
<td>Oral delivery</td>
<td>Feasibility studies in peptide drug develop...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral insulin</td>
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<td>Emisphere (Hawthorne, New York)</td>
<td>PODDS technology/selective binding of the drug to the transportable conformations in a non-covalent fashion. Once drug crosses membrane, complex dissociates and equilibrium resumes natural distribution so that most molecules are in therapeutically active state</td>
<td>Interferon, calcitonin, heparin, human growth hormone, insulin, parathyroid hormone</td>
</tr>
<tr>
<td>Flamel Technologies (Venissieux, France)</td>
<td>Micropump, an oral drug delivery system based on microencapsulation</td>
<td>Enables controlled delivery and prolonged absorption of protein drug in small intestine</td>
</tr>
<tr>
<td>Genentech (San Francisco, California)</td>
<td>Oral delivery</td>
<td>Recombinant human growth hormone</td>
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<td>Novartis (Basel, Switzerland)</td>
<td>Eudragit (methacrylic acid copolymers)/protease inhibitors</td>
<td>Drugs encapsulated in pH-sensitive nanoparticles and released at pre-programmed times</td>
</tr>
<tr>
<td>Nobex Corporation (Durham, North Carolina)</td>
<td>Amphiphilic polymers and covalent conjugation</td>
<td>Oral insulin, oral calcitonin, oral enkephalin, Oral insulin has completed phase II studies</td>
</tr>
<tr>
<td>Unigene Laboratories (Fairfield, New Jersey)/Warner-Lambert</td>
<td>Patented oral delivery technology for peptides</td>
<td>Oral calcitonin for osteoporosis in phase I/II clinical trials</td>
</tr>
<tr>
<td>Yissum Pharmaceuticals Inc. (Jerusalem, Israel)</td>
<td>Oral protein and peptide system to facilitate intestinal absorption using polymer technologies</td>
<td>Applicable to insulin and calcitonin</td>
</tr>
</tbody>
</table>
Site-Specific Mucoadhesives Using Lectins

Lectin Mediated Adhesion of Bacteria to Intestinal Mucus Gel or Cell Layer

Lectin-Mediated Mucoadhesion

- Mucus lining the epithelial cell layer contains many carbohydrates
- Proteins of non-immunological origin
  - Mainly found in seeds of legume plants
  - Specifically bind particular carbohydrates
- Mediate mucoadhesion with wheat germ agglutinin (WGA)

- 36 kDa, Binds N-acetyl-glucosamine and sialic acid
- Resists proteolytic degradation → minimal degradation in GI tract
- Low toxicity
Site-Specific Mucoadhesives Using Carbohydrate-Containing Copolymers


2. Fucose-Containing Copolymers: Higher Affinity to the Third Part of Jejunum

3. Cationic Copolymers: All Regions in the Intestine
Carrier Synthesis
Hydrogel Components

Methacrylic acid (MAA)

Poly(ethylene glycol) monomethylether monomethacrylate (PEGMMA)

Tetraethyleneglycol dimethacrylate (TEGDMA)

Cross linking agent

Irgacure® 184 (1-hydroxycyclohexyl-phenylketone)

Photoinitiator
Polymer Synthesis

1:1 MAA:EG (PEG-1000) 1mol% TEGDMA 1wt% Irgacure 184 50:50 wt% H₂O:EtOH solvent

Wash in D.I. H₂O for 7 days Vacuum dried for 2 days

UV initiated polymerization 16-17mW/cm² for 30 min.
Scanning Electron Micrographs

P(MAA-g-EG) microparticles crushed and sieved to <150 μm.
Carrier Functionalization

Step 1: Functionalize ACR-PEG-NHS with biotin-PEO-Amine → ACR-PEG-Biotin
ACR-PEG-NHS, MW 3400

Step 2: Polymerize ACR-PEG-Biotin with PEG and MAA → P(MAA-g-EG) PEG-Biotin

Step 3: Functionalize P(MAA-g-EG) PEG-Biotin with Avidin and Biotinylated-WGA

Confirmed 90% attachment of biotin to PEG via fluorescamine assay
MAA and PEG

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P(MAA-g-EG) PEG-biotin

Add biotinylated-WGA microparticles in PBS
Mucus Secreting Co-culture

• Co-culture Caco-2 cells with HT29-MTX cells, a sub-population of HT29 cells → want to create a more accurate in vitro model

• HT29-MTX cells
  – Colon carcinoma cells with ability to secret mucus
  – Form a polarized monolayer and develop an apical brush border
  – Secrete gel-forming mucins with carbohydrate moieties similar to small intestine

• Determine optimal co-culture conditions
  – Seeding ratio and density → TEER
  – Cell morphology, density, and mucus coverage → light microscopy and alcian blue staining
Intestinal Insulin Absorption

Plasma Insulin Levels

Hypoglycemic Effect

Plasma insulin level (mIU/mL)

0 60 120 180 240

Blood glucose level (% of initial)

0 60 120 180 240

P(MAA-g-EG)WGA

P(MAA-g-EG)

Insulin Solution

n = 3-5
Pharmacokinetic Analysis

<table>
<thead>
<tr>
<th>Carrier</th>
<th>$T_{\text{max}}$ (min.)</th>
<th>$C_{\text{max}}$ (μIU/mL)</th>
<th>AUC (μIU•hr/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(MAA-g-EG) WGA</td>
<td>10</td>
<td>313.3 ± 80.7</td>
<td>323.3 ± 103.1</td>
</tr>
<tr>
<td>P(MAA-g-EG)</td>
<td>10</td>
<td>380.9 ± 54.0</td>
<td>391.4 ± 117.9</td>
</tr>
<tr>
<td>Insulin Solution</td>
<td>120</td>
<td>2.22 ± 2.54</td>
<td>2.5 ± 1.7</td>
</tr>
</tbody>
</table>

Insulin dose = 25 IU/kg

$n = 3-5 \pm \text{SE}$
Residence Time

- The Gastric Emptying Time
  
  Gastric Emptying Time of Polycarbophil and Carbopol 934P Formulations was about 200 min.

- The Small Intestine Transit Time

  The Mean Transit Time of Polycarbophil and Carbomer (Carbopol 934P) Through the Whole Small Intestine was About 3 hours
Scintiscans Showing the Gastrointestinal Transit of Mini-Matrices in a Fed Volunteer.
Additional Material

See attached review article on mucoadhesive materials and relevant articles on our group’s successful mucoadhesive systems for oral protein delivery.