“An engineered cell-laden adhesive hydrogel promotes craniofacial bone tissue regeneration in rats”

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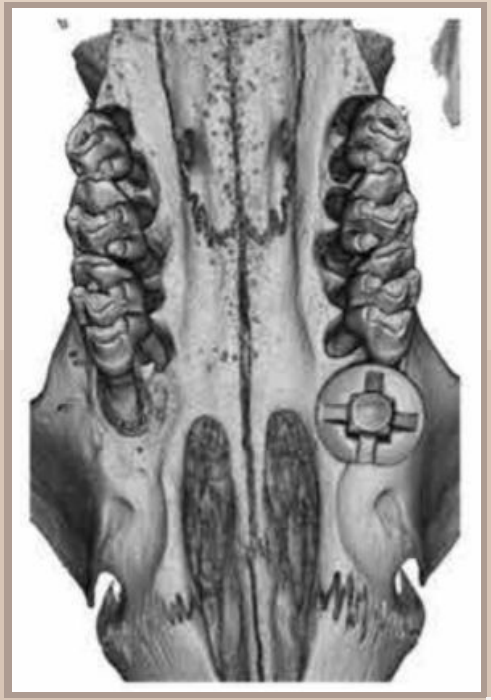


Opening Remarks

- **Basically:** An engineered adhesive allows for dental bone regrowth in rats
  - More specifically, Alginate-based adhesive, photocrosslinkable, and osteoconductive hydrogel biomaterial (**AdhHG**) with tunable mechanical properties

About the Authors:

- Mohammad Mahdi Hasani-Sadrabadi: Specializes in nanomedicine and immunoengineering, h-index of 32
- Sevda Pouraghaei: Specializes in nanotech and biomaterials, recent project in inner ear hair regeneration
- Song Li: specializes in cell and tissue engineering and is the chancellor's professor in the BE department!

Vocabulary

ADhHG: seen on last slide, main component (aka hydrogel) in the study

Aggregate: collection of particles forming a mass

MCS: Mesenchymal stem cells, cells (mostly in bone marrow) that can differentiate into a variety of cell types

GMCS: gingival (oral) mesenchymal stem cells

BBMCS: bone marrow mesenchymal stem cells, less favorable

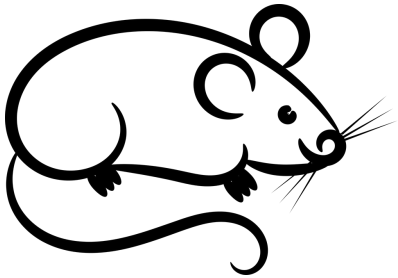
In vivo: taking place inside living organism

In vitro: taking place outside living organism

Peri-Implantitis: destructive inflammatory process affecting the soft and hard tissues surrounding dental implants

Introduction

- Bone grafts are often used in these types of procedures, but there are serious disadvantages
  - Mesenchymal stem cells (MSCs) become an alternate technique
- The lack of adhesion and retention in current methods (especially seen in oral methods)
  - L-dopa amino acid
  - Osteoconductive hydrogels
- Peri-Implantitis model was tested in rats, which confirmed injectivity and functionality



Discussion: Overview

- **Basically:** Engineered an adhesive hydrogel MSC delivery vehicle that is stable in the presence of saliva and the dynamic oral environment, and provides strong adhesion
- **Issue:** Existing techniques used to repair bone defects are inefficient and limited (painful, risky, high-cost, etc.)
- **Solution:** Administer MSCs via biomaterials as cell delivery vehicles
  - MCSs: Able to differentiate into many different types of cells; extensively distributed in many adult tissues
- Using biomaterials as cell delivery vehicles require **adhesion and retention** of the biomaterial at the application site

Discussion: Overview

- **Issue:** Previous cell-laden biomaterials for periodontal (structures surrounding/supporting the teeth) regeneration:
  - Do not adhere well to the tissue
  - Possess poor mechanical strength
  - Degrade quickly and uncontrollably
  - Do not possess thorough regenerative properties
- **Solution:** The hydrogel developed in this study:
  - Can be delivered in a minimally invasive manner
  - Adheres strongly to the surrounding orofacial tissues
  - Provides sites for cellular attachment
  - Possesses favorable mechanical properties
  - Possesses a desirable degradation rate

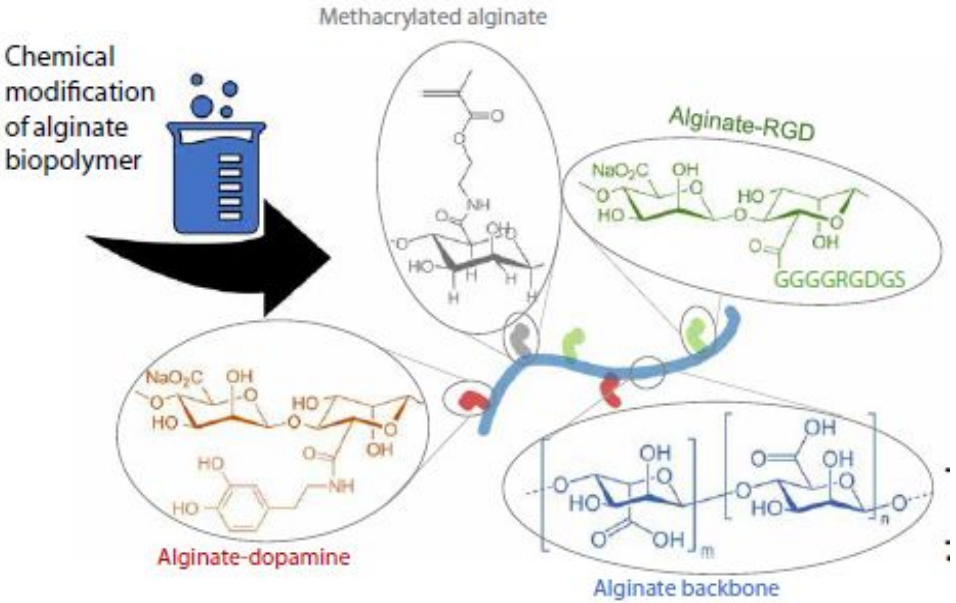
Discussion: Application

- **How to Apply:** Inject into bony defeat site through application of shear stress during injection; hydrogel readily fills defect site
- **How to Stabilize:** Quickly set through photocrosslinking; further stabilized by applying visible light to induce chemical cross-linking with surrounding tissues (promotes retention and enhances mechanical properties)
- **Cell Vehicle:** Hydrogel delivers dental-derived stem cells to induce bone regeneration

Results

- The hydrogel described in this paper was created from alginate, which is found in **algae**!
- Alginate has been used previously to encapsulate cells and fragile bioactive materials.
- Alginate can be formed into a scaffold to place the MSCs in the most optimal arrangement
- The ability to arrange the MSCs in this way is important because it allows for various environments to be closely mimicked

Adhesive hydrogel synthesis and characterization



1. Used existing methods to synthesize methacrylated alginate hydrogel

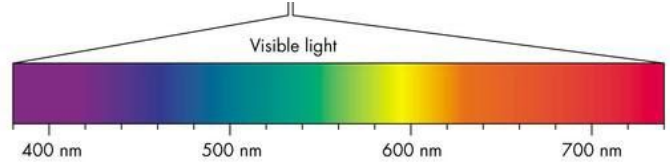
2. To improve the adhesive properties if the alginate, used carbodiimide bioconjugation to incorporate DA hydrochloride.

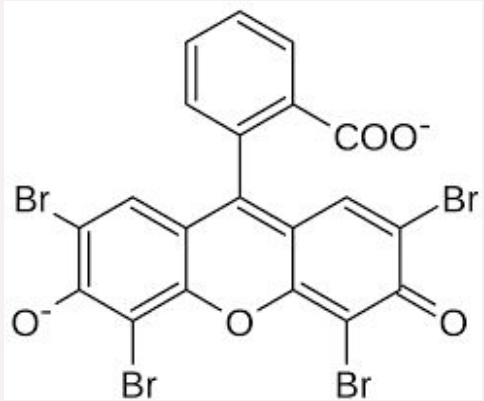
3. The alginate-based adhesive hydrogel (AdhHG) was produced by modifying the
resulting hydrogel with a collagen-mimicking short peptide [(Gly)4-Arg-Gly-Asp-Gly-Ser; RGD].

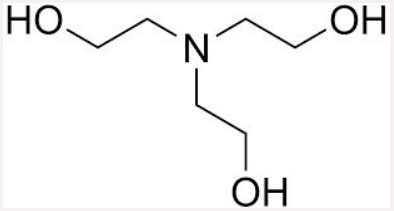
4. AdhHG was cross-linked using a Ca^{2+} rich-media or by oxidizing DA residues.

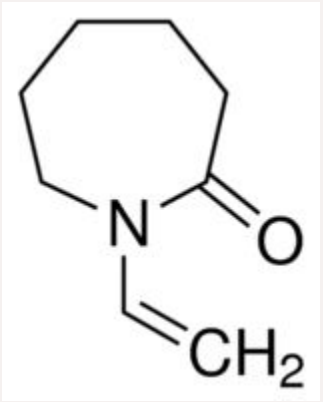
5. In order to initiate a photopolymerization reaction, 3 compounds were needed: eosin Y, triethanolamine, and vinyl caprolactam.

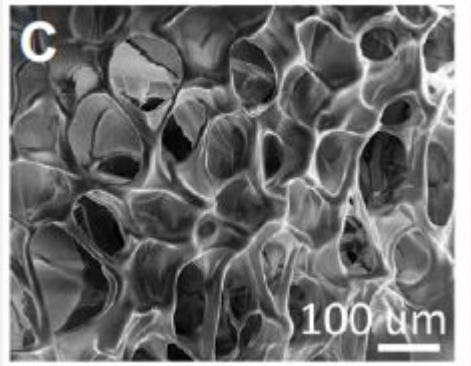
6. Visible light in the 450-550 nm range was used. The material was subjected to 100mW/cm^2 for 20s.

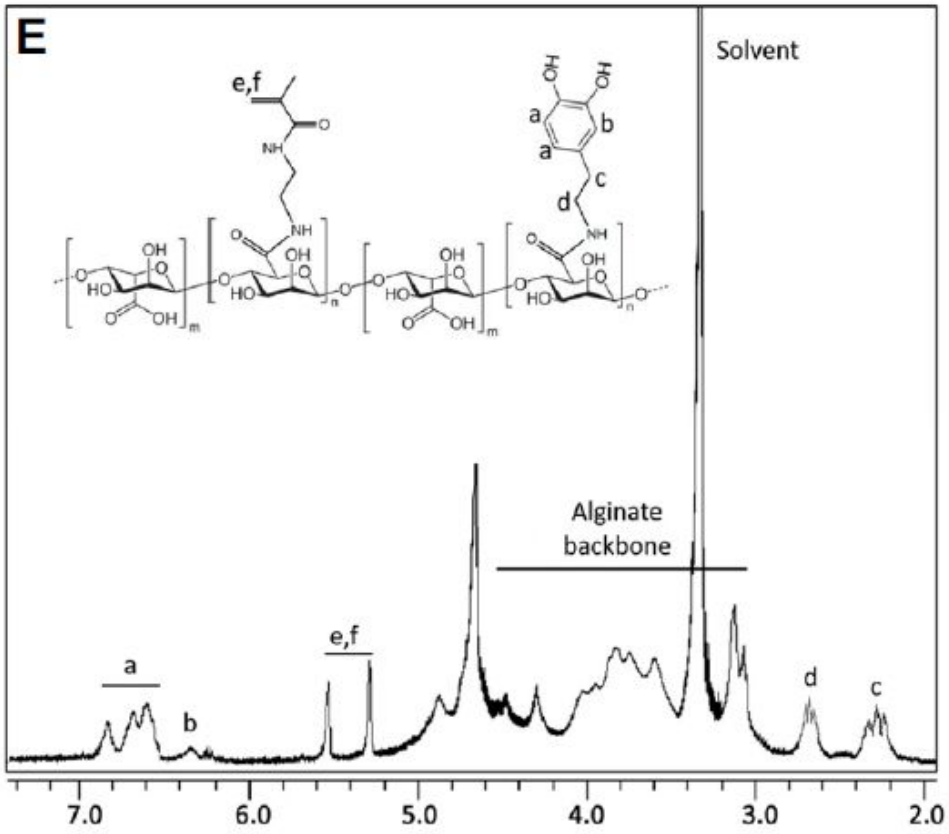






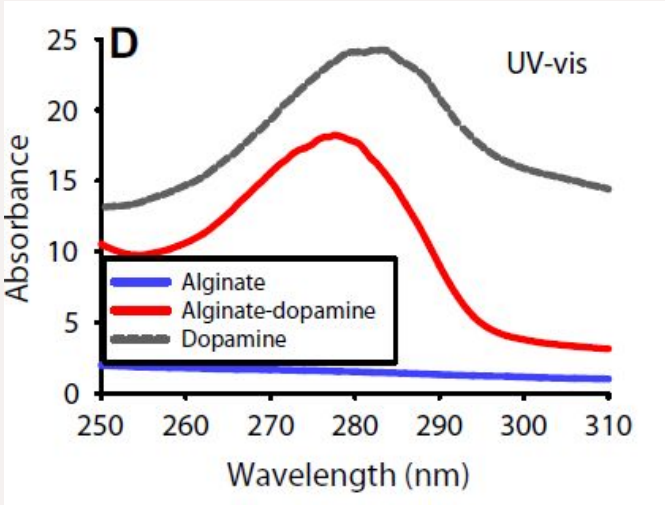






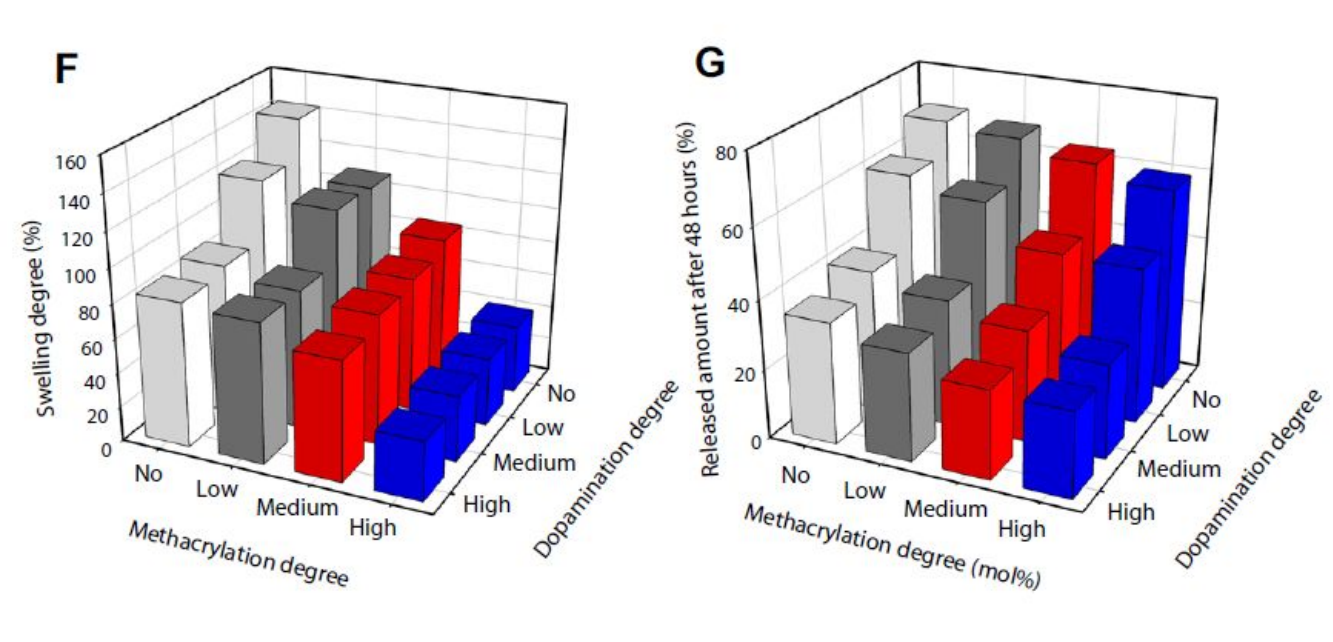
NMR = Nuclear Magnetic Resonance

- A peak at 278nm in the UV visible spectroscopy plot indicates that the DA residues are present in the hydrogel
- The researchers used NMR to verify that the alginate was successfully modified with DA and methacrylate residues



These plots indicate that the adhesive hydrogel system has properties that can be adjusted by varying the degree of methacrylation and dopamination.

The photoinitiator concentration and exposure time are both factors included in the degree of dopamination.



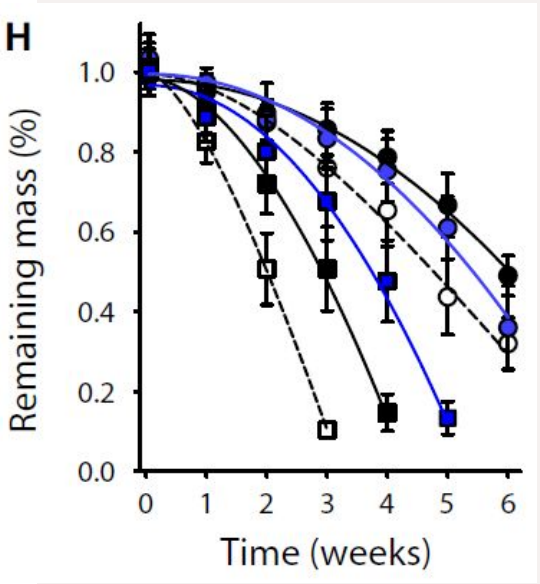
Elasticity & Reproducibility

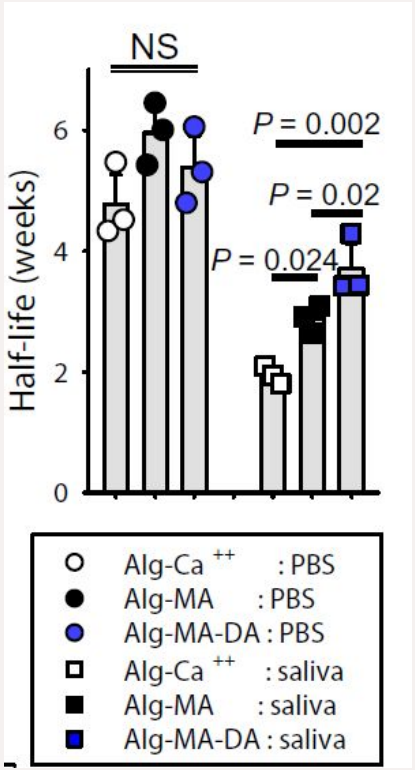
- The researchers designed the hydrogel to have an elastic modulus between 22 and 30 kPa.
- To confirm that this elastic modulus could be achieved repeatedly, samples of the hydrogel were synthesized 6 different times over the course of 3 months
- There were no statistically significant differences in the mechanical properties amongst the various batches.
- It was also determined that batches of hydrogels that were freeze dried had a shelf life of up to 2 years after being synthesized

In Vitro Biodegradation

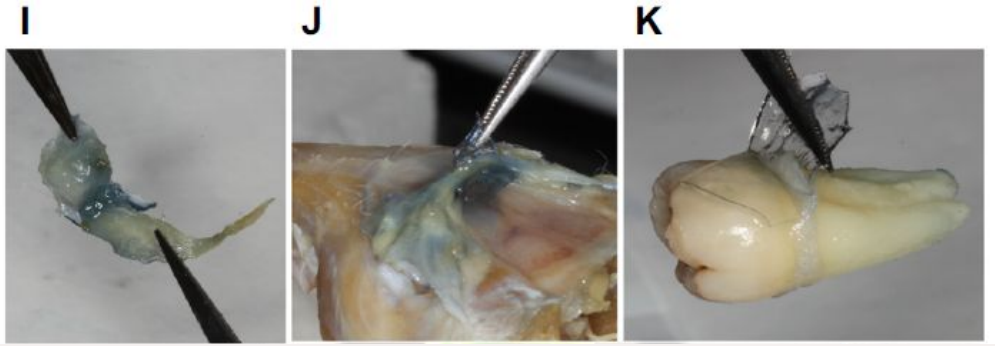
In order to measure how the material would hold up under real physiological conditions, the various hydrogels were subjected to either phosphate buffered solution (PBS) or human saliva at 37°C.

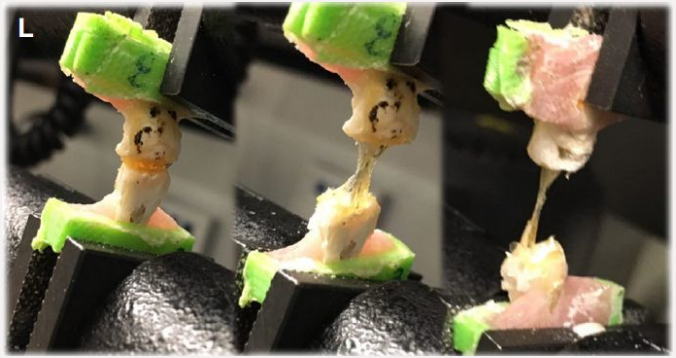
The mass of the hydrogels was tracked over time, which allowed for the half-lives to be determined for the different hydrogels.

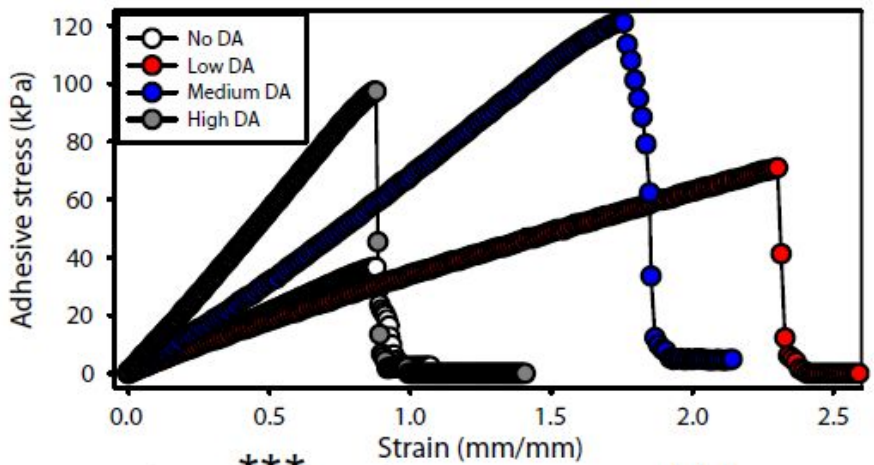


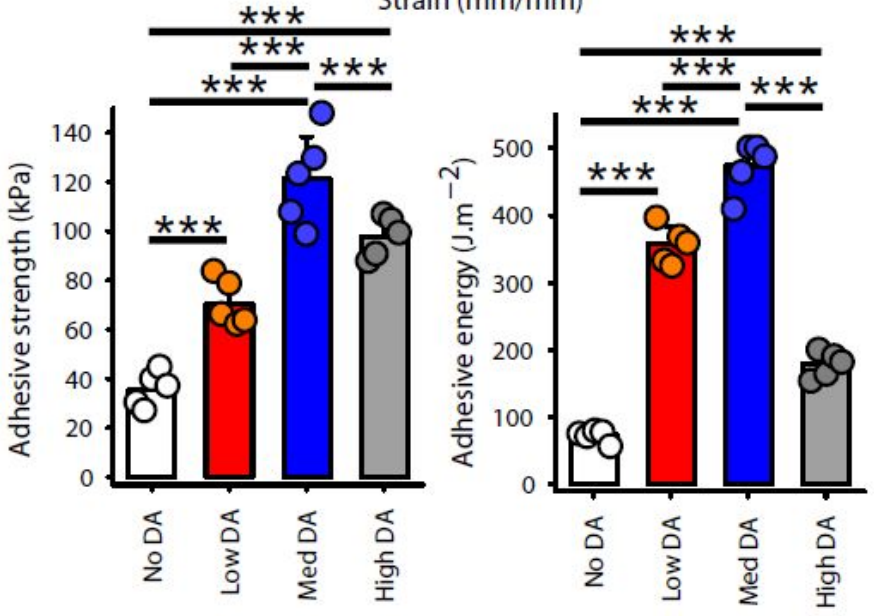


- For craniofacial bone regeneration to be successful, the biomaterial containing the cells must be able to adhere and retain well to the desired site
- Gingival tissue (I), alveolar bone,(J) and teeth (K) were used to evaluate the adhesive properties of the hydrogels.
- Crosslinked AdhHG adhered to both strong and hard tissues
- Hydrogels prepared without DA residues were unable to adhere to any of these substances.
- Additional testing was conducted to determine how different levels of DA conjugation impacted adhesive properties (L)

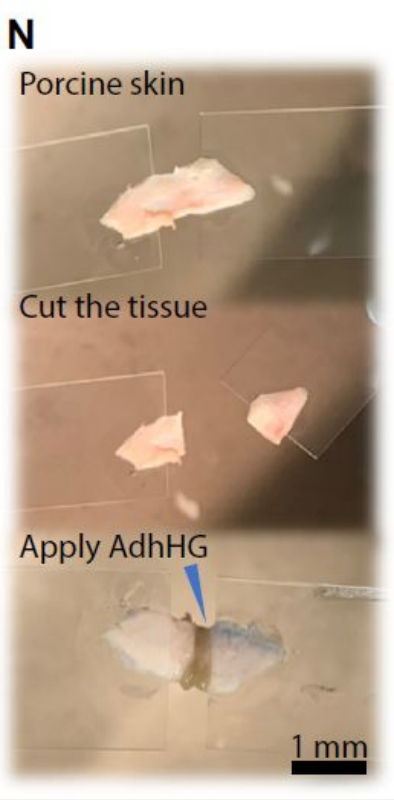




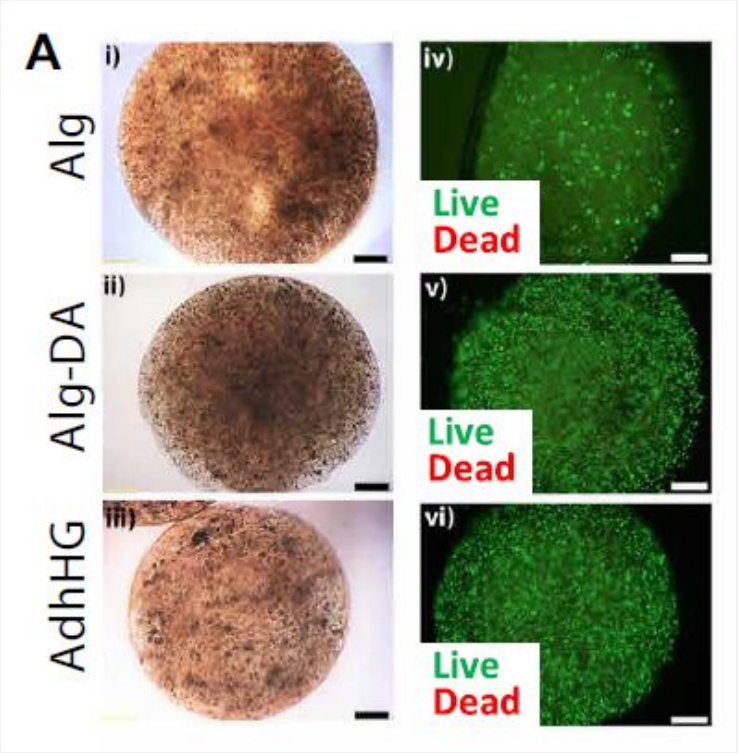


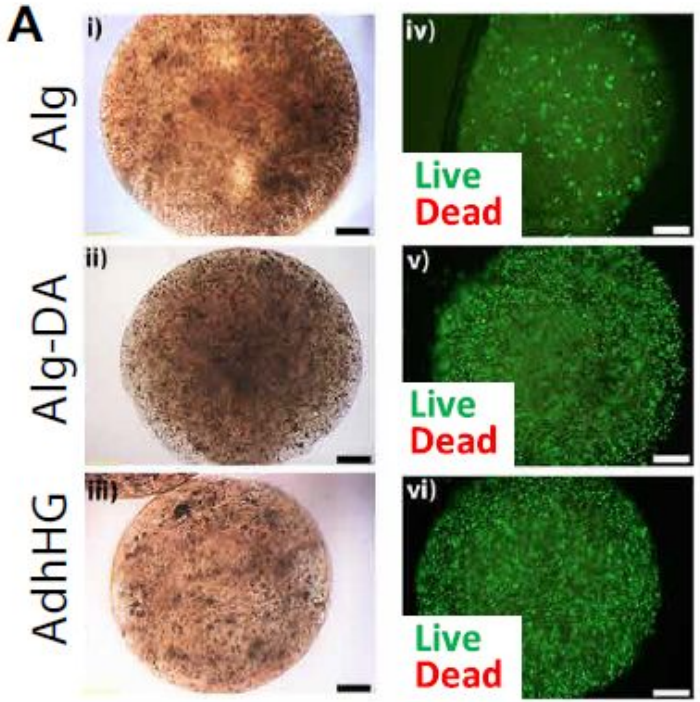


- The hydrogels with medium amounts of DA conjugation were able to withstand the most stress when applied to porcine gingival tissue
- DA causes strong intermolecular and intramolecular H-bonding.
- At a certain point, having more DA becomes disadvantageous because the high number of H-bonds causes the hydrogel to become brittle



In vivo biocompatibility and biodegradation of adhesive hydrogels

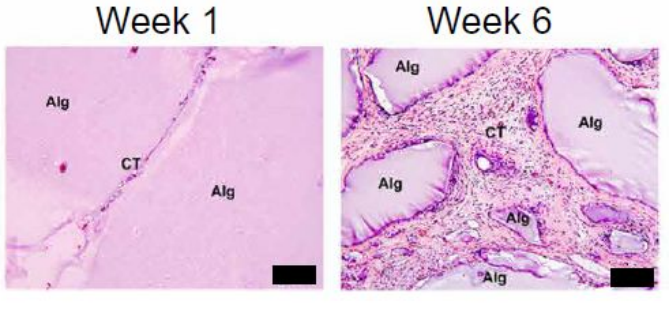




- The researchers selected giniviginal mesenchymal stem cells (GMSCs) over bone marrow mesenchymal stem cells (BMMSCs) for use in the engineered scaffolds due to their better growth properties.
- These cells were checked for surface markers that identify GMSCs confirmed to be negative for markers that identify hematopoietic stem cells
- The figure shows hydrogel spheres encapsulating the GMSCS at a density of 2x 10^6 cells/mL

In Vivo Biocompatibility

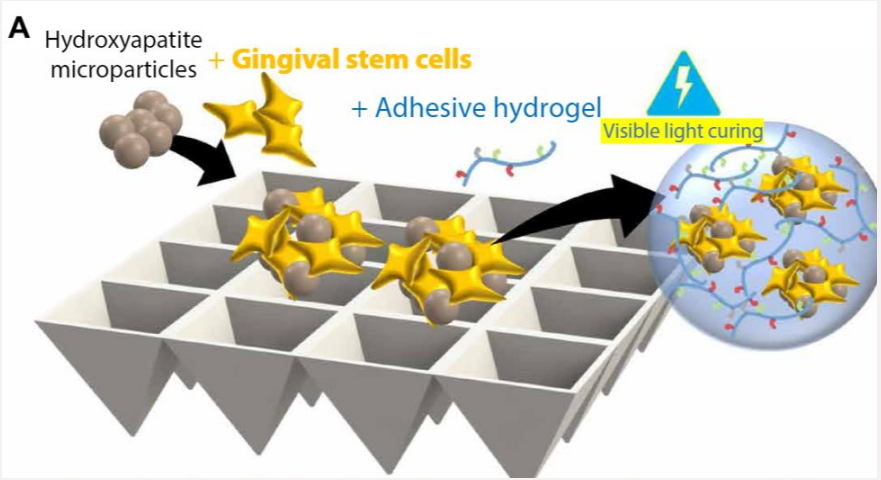
- Wild-type mice received the hydrogel spheres via a subcutaneous implantation.
- After 7 days, no lymphocyte or macrophage infiltration was observed.
- The mice's blood was tested to determine if that hydrogel was potentially toxic.
- There were no statistically significant differences in the blood response to this novel hydrogel and commercially available Alginate RGD hydrogels
- Metabolic screening indicated that there were no significant changes in kidney or liver functions, although there were some changes in electrolytes observed



In Vivo Degradation

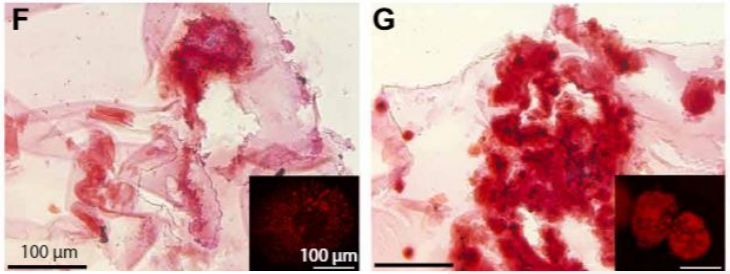
- Wild-type mice received hydrogel spheres with optimized adhesive properties via a subcutaneous implantation.
- The degradation of the hydrogels was tracked over a period of 6 weeks.
- After this time more than half of the alginate had dissociation.
- The biodegradation rate can be adjusted by changing the molecular weight of the alginate back bone through controlled oxidation.
- When the backbone is highly oxidized the material will be absorbed within a month, while hydrogels with lower oxidation levels should stay intact for more than 6 weeks.

In Vitro Osteogenic Differentiation of MSC Aggregates Encapsulated in Adhesive Hydrogels

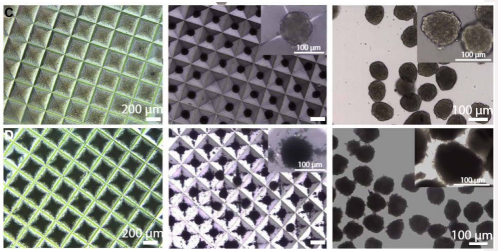


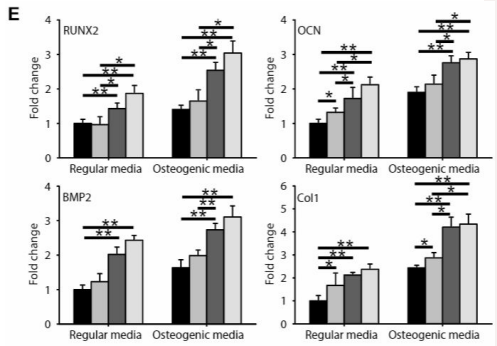
Results

- Goal: Create a hydrogel that is osteoconductive yet free of growth factors
  - Achieved by modifying hydrogel with HAps MPs
    - Enhance shear-thinning properties
- Developed stem-cell aggregates incorporated with HAps MPs.
  - Then encapsulate aggregates in AdhHG
    - Controls factors of extracellular environmental cues to direct MSC differentiation
    - Mimics cell contact in bone tissue to induce osteogenesis



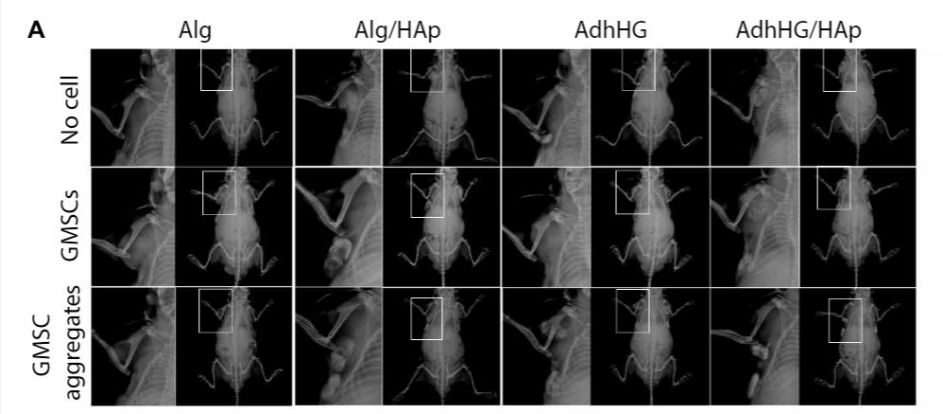
Results



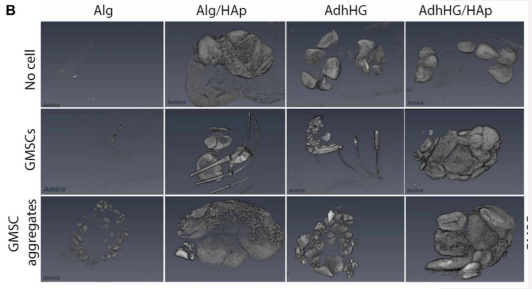


- Results
  - HAp particles induced osteogenic differentiation in human GMSC
  - Enhanced mechanical properties
  - Strengthened interfacial properties
- Each cell aggregate contained 800-1200 GMSC
  - Ratios of 1:2, 1:1 and 2:1 HAp MPs were tested
    - Viability and metabolic activity not affected by ratio
    - Since ratio doesn't affect outcome, used 1:1 ratio
- GMSC and aggregates maintained their viability for 7 days after injection

In Vitro Regenerative Properties of Adhesive Hydrogels

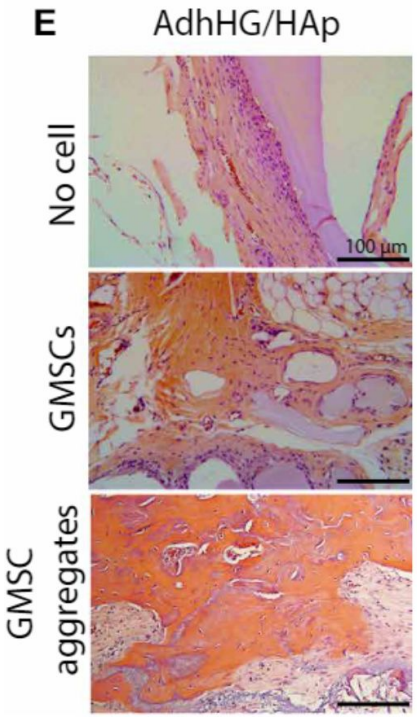


Results



- Used ectopic bone formation model to test the intrinsic osteogenic properties
  - Immunocompromised mice were injected with either cell free or cell aggregate laden hydrogel
  - Mice were examined after 8 weeks
- Peri-implantitis (inflammation and bleeding at implantation site) was induced in mice
  - Also causes bone loss
  - Hydrogel was injected at these sites
    - Assessed at 4 and 8 weeks

Results



- Results
  - X-ray of whole mouse confirmed ecoptopic osteogenesis and revealed stem cell-laden hydrogels formed nodules similar to natural bone
- Even without HAp MPs, Da residues formed but cell aggregates produced significantly more.
- Not a significant difference in volume, but significant difference in density
  - Bone-like tissue was more highly mineralized and denser when produced by cell aggregates.
- Bone regeneration was significantly greater in mice after injection of hydrogel laden with GMSC.

Methods & Materials: Study Design

1. Engineer AdhHG base
2. Assess safety of AdhHG in mice
3. Confirm physical and biological properties of the AdhHG
4. Fabricate GMSC aggregates with incorporated HAp MPs and encapsulate them in the ADhHG
5. Evaluate efficacy of ADhHG with HAp MPs in regenerating bone tissue
    a. Implant inoculated dental implant into rats to generate peri-implant bone loss and inflammation
    b. Inject rats with engineered hydrogels to regenerate lost bone
    c. Quantitatively assess inflammatory profile via expression of inflammatory and anti-inflammatory cytokines at different time intervals

Methods & Materials: Study Design cont.

- *In vitro* experiments were performed 3 or 4 times
  - $n = 5$ (random assignment) for each experiment
- *Ex vivo* and *in vitro* data were not blinded; experimenter was blinded to analysis of all animal-related samples

Methods & Materials: MSC Confirmation and Isolation

Purpose: To confirm MSC characteristics of cells in GMSCs and isolate GMSCs for encapsulation in AdhHG

- 10 healthy male or female 16- to 22-years-olds undergoing third molar extractions
- Extracted subjects' gingival tissue to isolate and culture their GMSCs
- MSC characteristics were analyzed via flow cytometry to confirm that they were positive for MSC surface markers and negative for hematopoietic cell markers
- GMSCs were encapsulated in AdhHG

Methods & Materials: Synthesis of AdhHG

Purpose: To synthesize AdhHG to further modify for future use

1. Oxidize and purify alginate; recover purified alginate by freeze-drying the solution
2. Synthesize alginate-DA by activating the carboxyl groups of alginate and reacting them with the amino groups on DA
    - Confirmed via UV-visible and NMR spectroscopy
3. Prepare methacrylated alginate (Alg-DA-MA) by reacting Alg-DA with 2-aminoethyl methacrylate hydrochloride (AEMA)
    - Methacrylation and DA conjugation degrees were varied to study their effects on biophysical properties of the resulting hydrogels

Methods & Materials: Synthesis of AdhHG cont.

4. Synthesize AdhHG by coupling amine-terminated peptide to the carboxylic groups of alginate via EDC/NHS chemistry
5. Lyophilize and store synthesized AdhHG at -80°C
    - Store some samples at 22°C for more than 3 months to examine shelf-life stability
6. Integrate HAp MPs to GMSC aggregates to engineer an osteoconductive adhesive hydrogel
    - Add different ratios of HAp MPs to the GMSC aggregates
7. Mix GMSC aggregates with AdhHG to form cell aggregate-laden hydrogels
    - The HAp density and loading content of the hydrogels are the same despite the different ratios

Methods & Materials: Synthesis of AdhHG cont.

8. Culture GMScs in AdhHG in standard or osteogenic induction medium for 4 weeks
    - Examine osteogenic potential of encapsulated GMSCs through Xylenol orange staining and qPCR of osteogenesis-related genes in GMSCs to detect HAp MPs

- 6 batches of 5 hydrogels each were synthesized to examine batch-to-batch variation

Methods & Materials: Evaluation of Physical Properties of Hydrogels

Purpose: To physically characterize the hydrogels

- Mechanical Properties (Elasticity)
- Injectability
- Rheological (Deformation) Testing
- Swelling Kinetics
- Microstructure Effect on Permeability
- Microstructure

Methods & Materials:
In vitro cytocompatibility studies

- 1×10^6 to 1×10^8 cells/ ml
- 2D cell seeding on the hydrogel surface
  - Incubated for 14 days
  - Media change twice per week
- 3D cell encapsulation within the hydrogel
  - Trypsinized cells
  - Resuspended in alginate hydrogel w/ photoinitiators
  - Cell-laden alginate hydrogels cured
  - Washed 3x w/ PBS
  - Incubated for 14 days in standard SC culture medium

Methods & Materials:
In vitro cytocompatibility studies

- Cell Viability
  - calcein-AM/ethidium homodimer Live/Dead Assay
- Biodegradation
  - Hydrogel in PBS at 37 ℃
  - Hydrogel in human saliva at 37 ℃
  - Weight loss measured at (8 hrs, 1, 2, 3, 4, 5, 6 weeks)

Methods & Materials: Ex vivo adhesion tests

- Rat/pig/human gingival, alveolar bone, and tooth root
  - Samples cut (2x2x2 mm) then cut in half
  - Immersed in PBS
  - Embedded in 3D-printed ABS blocks (10 x 30 mm) or glued to glass slides
  - Two samples tested for tensile loading
    - Hydrogel added and cured to adhesive area
    - Adhesion determined at point of detachment

Methods & Materials:
In vitro osteogenic differentiation assay

- Test effects of cell aggregates and HAp MPs
  - Aggregates formed using force aggregation technology
  - 1:1 HA/GMSC ratio
  - GMSCs were encapsulated in adhesive hydrogel & cultured in an osteogenic media
  - Alginate microspheres without cells used as negative control
  - Staining after 4 weeks w/ xylenol orange
  - BMP2, OSX, BSP, OPN, RUNX2, OCN, and Col I (osteogenic genes) was assayed by qPCR analysis

Methods & Materials:
In vivo biodegradation and biocompatibility analyses

- Approved mice model
- Samples injected in subcutaneous implantation
- Two materials tested, alginate-RGD and the alginate based hydrogel
- Follow up on 3, 7, 14, and 28 days
- Removed w/ surrounding tissue at 7, 14, 28 days.
- Immunohistological and histological tests performed
  - Biocompatibility determined by presence of inflammatory factors
    - Lymphocytes (CD3) and macrophages (CD68)
  - Histological evaluation of scaffold at 7, 14, 28 days used to determine degradation

Methods & Materials:
GMSC-mediated bone formation: Subcutaneous model

- Ex-vivo GMSC aggregates w/ HAp MPs encapsulated in hydrogel
- Subcutaneous implantation at dorsal surface of 5-month old mice
- Euthanized after eight weeks
- Radiographic examination, micro-CT analysis, and H&E staining used to evaluate bone regeneration

Methods & Materials:
Peri-implantitis model

- Thirty 2-month-old Sprague-Dawley rats used
- Used titanium implants to introduce strain of A. actinomycetemcomitans biofilm to induce peri-implantitis transmucosally
- Three weeks after surgery, appearance of tissue characteristic of peri-implantitis
- Then the hydrogel was injected at the periphery of the implants and polymerized w/ visible light.
- Groups of animals were euthanized at 2, 4, 6, & 8 weeks after injection.
- Radiographic examination and micro-CT used to assess bone regeneration

Discussion: Limitations

- Lack of real-time *in vivo* assessment of hydrogel degradation and new bone formation
- Further optimization is required to manipulate interaction with the immune system in the damaged tissue to promote tissue repair
- Designed hydrogels have a limited crosstalk with immune cells
- Investigations have been performed on small animals (requires larger animal models before moving toward clinical translation)

Closing Remarks

- **Problem:** cell-laden hydrogels for bone tissue regeneration are not optimized for use in the oral environment
- **Solution:** Alginate-based (algae-inspired) adhesive with tunable mechanical properties and biodegradability

- **Application:** Use of adhesive as an MSC delivery vehicle to promote damaged bone regeneration for craniofacial tissue engineering applications
- Findings encourage future use of engineered biologically active and adhesive biomaterial for bone tissue engineering

Work Cited

1. M. M. Hasani-Sadrabadi, et al., An Engineered Cell-Laden Adhesive Hydrogel Promotes Craniofacial Bone Tissue Regeneration in Rats. *Sci. Trans. Med.* **12**, eaay6853 (2020).

Questions?

Kahoot time!