

RHD

CONFERENCE POSTERS

2016 ASGCT

This is one of the most recent scientific poster that I presented at last year's American Society of Gene and Cell Therapy in Washington DC. My postdoctoral research is on bioprocess development for genetically engineered stem cells.

genetic engineering

Non-viral tissue engineering of clinically-relevant primary cells in stirred suspension bioreactor

cellular reprogramming

induction of pluripotency or direct conversion in a scalable format

automation

process can be automated in parallel for multiple custom small-run orders.

integrated platform

streamline the derivation and expansion of engineered cells as one vertically integrated process

NON-VIRAL TRANSFECTION OF PRIMARY HUMAN FIBROBLASTS ON MICROCARRIER SUSPENSION CULTURE

Charlie Y. Hsu and Derrick E. Rancourt

Introduction

Suspension bioreactors have been employed for the large-scale production of recombinant proteins and expansion of cells for clinical applications. However, the process requires a stably-expressing cell line to begin with, which can take considerable amount of time and cost to establish. A transient-expression system not only provides a simple cost-effective platform for production in a small-medium scale, but is better suited for cellular reprogramming and tissue engineering of primary cells where only transient forced expression of factors is needed.

Here, we describe an efficient non-viral method to transfect primary human foreskin fibroblasts on suspension microcarrier using cationic reagent. This is the first step towards the development of an integrated platform to streamline the derivation and production of reprogrammed cells.

Methods

Inoculating Microcarrier

Microcarriers were first dispensed onto a polyHEMA-treated TC-dish, then single-cell suspension of fibroblasts were added to a final concentration of 100,000 cells per 10 mg of microcarriers per ml of culture media.

Growth Curve

Cell proliferation on the microcarriers were determined using the MTT assay. Each time point were expressed as a percentage over a mitomycin C-treated static fibroblasts culture

Transfection

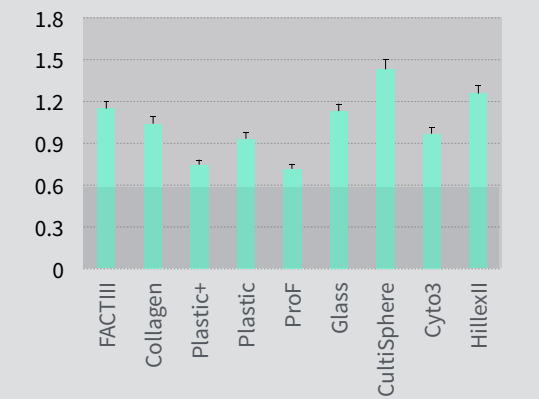
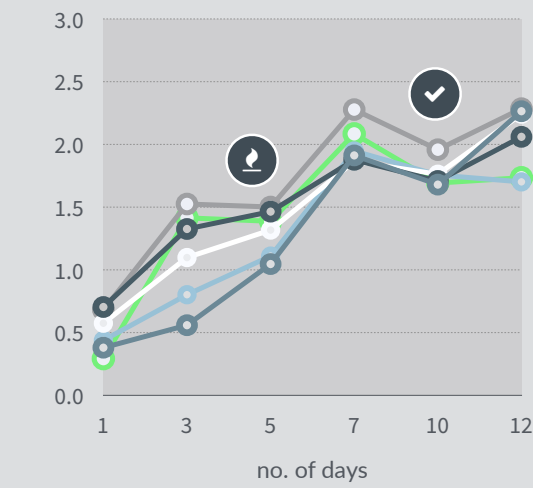
Cells were transfected on microcarrier in static suspension using XtremeGENE-HP. Two days after transfection, cells were then dissociated by 0.25% trypsin and processed for analysis by flow cytometry.

Microcarrier

Microcarrier	Size (µm)	Surface
Hillex II modified polystyrene	160-180	+
Glass cross linked polystyrene	125-212	+
Pronectin F cross linked polystyrene	125-212	⚡
FACTIII cross linked polystyrene	125-212	+
Cytodex 3 cross-linked dextran	175	+
CultiSphere S modified polystyrene	130-380	/

Growth Curve

A dip in the growth curve on Day 5 indicate media exhaustion. By Day 7, most of the microcarrier culture reached a plateau in cell density.



Cell Attachment

Attachment efficiencies differ among microcarriers, which translate to differences in starting cell density. This, in turn, would affect growth rate, time frame for transfection and ultimately, transfection efficiency

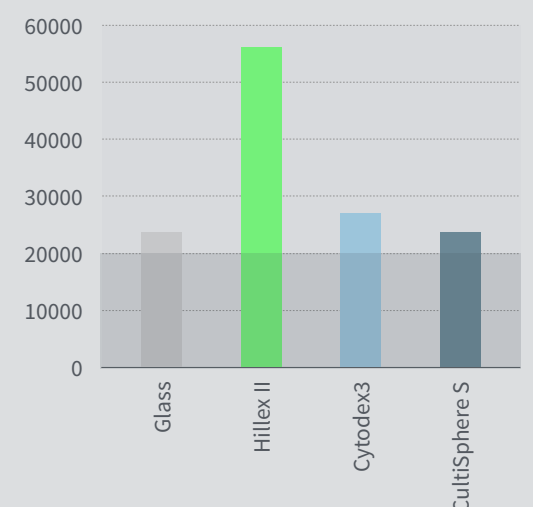
Cell Proliferation

Polymer-assisted transfection is highly dependent on the proliferation rate of cells. The optimal time frame to transfect should be around when the growth curve exhibit the sharpest positive slope.



Percentage of Transfection

The percentage of cells transfected were about the same among microcarriers. Interestingly, this approximately corresponds to the percentage of cells in the mitotic phase (18-20%)



Transfection Efficiencies

Despite having similar percentages of transfected cells, the levels of transgene expression differ significantly among carriers; Hillex II was the most conducive for high transgene expression

02.1

01

02.2

03.1

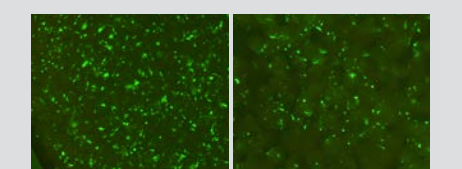
04

03.2



Transfection Time Frame

Transfection in static 2D culture is typically done at a certain cell density since cell-to-cell contact is necessary in driving the proliferation of adherent cells. Cell density on microcarrier is less straightforward to gauge, and therefore, difficult to assess culture readiness for transfection. Here, we transfected at multiple time points, bracketing around the exponential part of the growth curve.



Transfected cells on microcarrier

Representative epi-fluorescent images of HFF transfected with GFP on Cytodex 3 (left) and CultiSphere S (right).

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Another poster that I presented at last year's American Society of Gene and Cell Therapy. I like to use these posters as a creative outlet to try out some new designs

I did a split panel design so I can cut it in half and put it in checked luggage instead of having to carry a giant tube as carry on.

En route to non-viral genetic engineering:

Kinetics of pDNA uptake and transgene expression following repeated transfection with multiple episomal plasmids in primary human fibroblasts

Charlie Y. Hsu and Derrick E. Rancourt

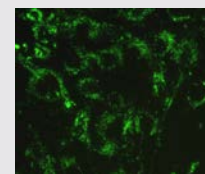
Introduction

Non-viral approach to genetic engineering of mammalian cells often involve co-transfection with multiple types of nucleic acid molecules. A significant rate-limiting step thus lies in the lack of efficient co-transfection, in which a subset of the transfected cells may be devoid of either one, two, or more of the factors required.

In this study, we used a multiplexed approach to examine the kinetics of DNA uptake and transgene expression following co-transfection with multiple fluorochrome-labeled reporter plasmids.

Methods

Non-invasive fluorescent labeling of plasmid DNA



Plasmid DNAs were labeled using the Mirus Bio Label IT® Cy3 and Cy5 Nucleic Acid Labeling Kits according to manufacturer's protocol. However, in order to measure transfection efficiency from the labeled plasmid,

we titrated labeling densities against uptake and transfection efficiencies. Figure A shows that the signal intensity is proportional to the amount of labeling reagent, while uptake and transfection efficiency (%Cy5 and GFP level) is inversely correlated (Figure B and C).

Transfection of human foreskin fibroblast

Primary human foreskin fibroblast were transfected with Cy5-labeled gWiz-GFP and Cy3-labeled gWiz-BFP. A 0.02 (v/w) Cy5-to-DNA labeled gWiz-BFP were mixed with a 0.1 (v/w) Cy3-to-DNA labeled gWiz-BFP at a 1:1 (w/w) DNA-to-DNA weight ratio, then transfected using XtremeGENE HP at a reagent-to-DNA v/w ratio of 3.

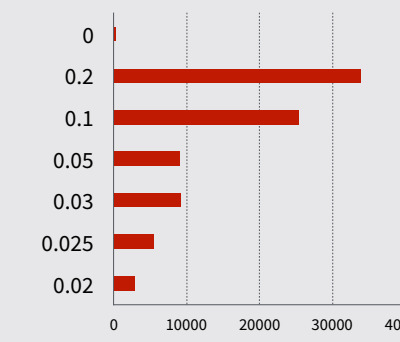


Figure A. The overall mean fluorescence (RL1) of cells transfected with plasmids labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

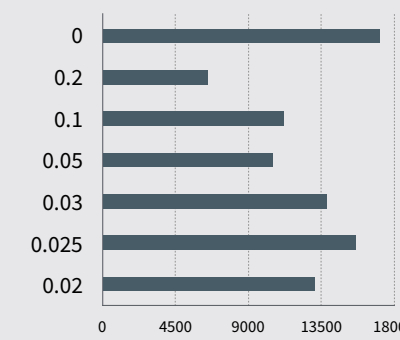


Figure B. The overall mean fluorescence (BL1) of cells transfected with gWiz-GFP labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

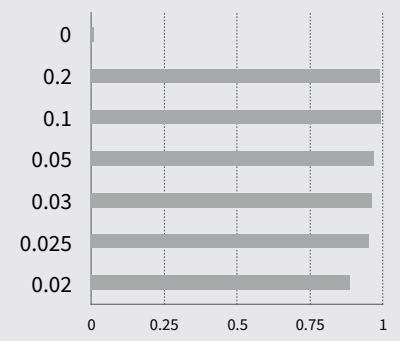
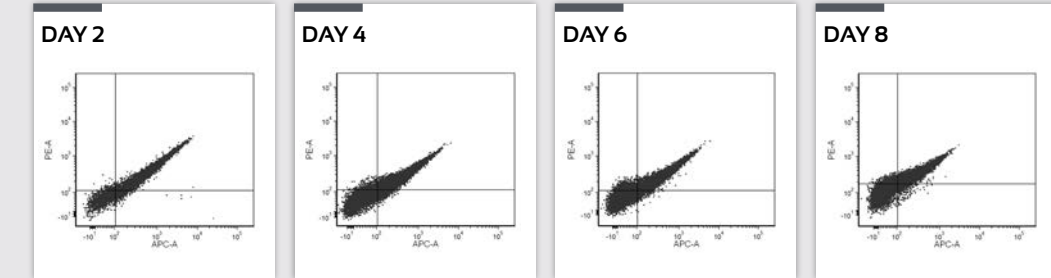


Figure C. The % of Cy5+ cells following transfection with plasmids labeled with decreasing ratios of Cy5-labeling reagent-to-pDNA (v/w).

RESULTS

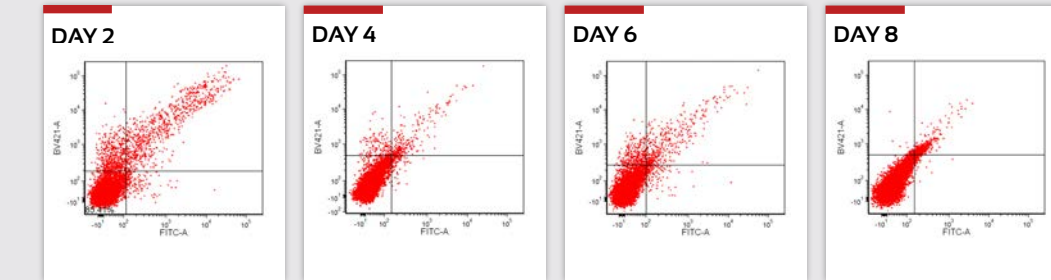
Kinetics of co-pDNA uptake

Figure 1. Fluorescent intensities of Cy5 and Cy3 over a course of 8 days



Kinetics of co-transfection

Figure 2. Fluorescent intensities of GFP vs BFP over the course of 8 days.

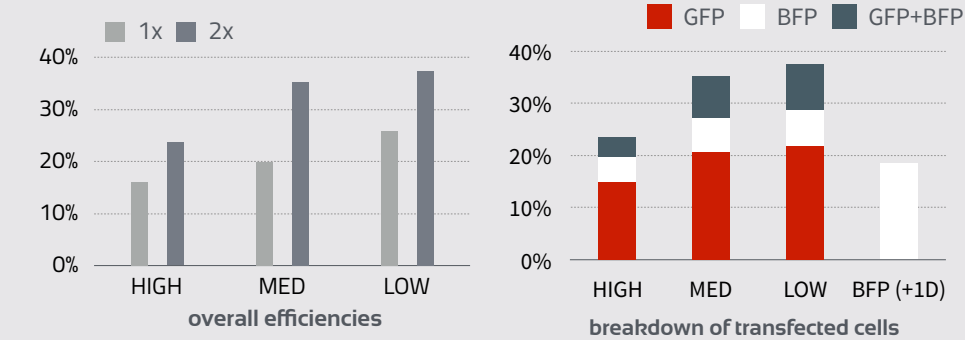


While majority of the transfected cells co-express both BFP and GFP, small proportions were singly transfected with either BFP or GFP. Further, the relative levels of transgene expression were not equivalent; some cells had more BFP than GFP, and vice versa.

We then looked at the effects of repeated transfection by first transfecting with BFP then with GFP. To our surprise, the increase in overall transfection efficiency came from new events, not pre-existing events, suggesting transfected cell may be refractory to subsequent rounds of transfection.

Repeat Transfection

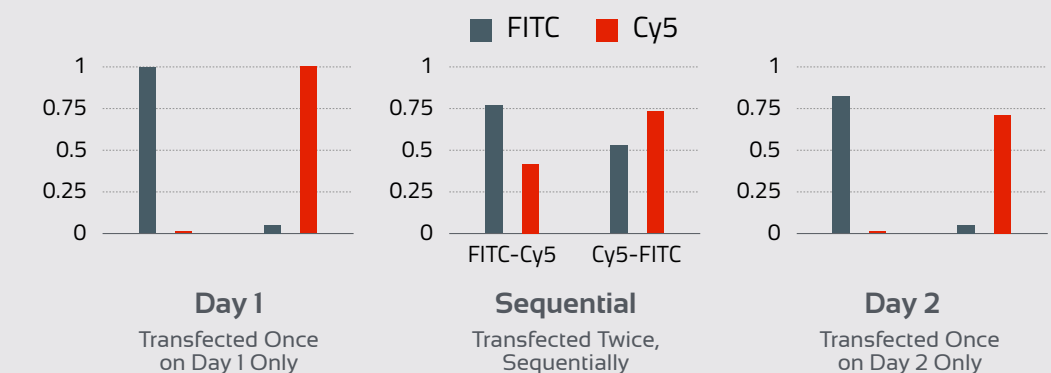
Figure 3. Transfection efficiencies following sequential transfection



We then looked at whether cells were refractory to even DNA uptake. By transfecting cells sequentially with two different fluorochrome-labeled plasmid DNA, we saw that there is indeed a reduction in the capacity to take up DNA.

Sequential Transfection

Figure 4. DNA uptake following sequential transfection



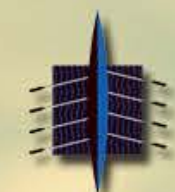
Summary

Co-transfection with multiple plasmid DNAs invariably results in asymmetry in the levels of transgene expression. Over time, the differences in relative expression levels widens, in which the stoichiometric ratios of the expressed factors deviates significantly from the intended input weight ratios.

In the context of cellular reprogramming where pluripotency-associated transcription factors are delivered separately by multiple episomes, this presents a major rate-limiting step. This may in part, explain why some iPSC-like colonies appear to be partially reprogrammed while only a few commit to full pluripotency.

PROMOTION POSTERS

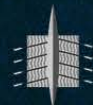
This poster was for the Alberta Rowing Association, to recruit athletes for the Own the Podium campaign for the Canada Summer Games



ALBERTA ROWING ASSOCIATION PRESENTS PODIUM PROJECT

Alberta Rowing Association and the University of Calgary are looking for athletes to become the next generation of provincial, national and Olympic champions

ARE YOU BETWEEN THE AGE OF 17 AND 22 YEARS OLD?
HAVE COMPETED IN SPORTS AT A CLUB/PROVINCIAL/NATIONAL LEVEL?
ARE MENTALLY TOUGH AND COMPETITIVE, ARE QUICK, AGILE, POWERFUL, FIT AND STRONG?
INTERESTED IN BECOMING PART OF CANADA'S MOST SUCCESSFUL SUMMER OLYMPIC SPORT?
If you think you have what it takes, please contact Sarah at sarah.laing@calgaryrowing.com



ALBERTA ROWING ASSOCIATION PRESENTS **PODIUM PROJECT**

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Are you between the age of 17 and 22 years old?

Have you competed in sports at a club/provincial/national level?

Are you mentally tough and competitive?

Are you quick, agile, fit, powerful and strong?

Are you interested in becoming part of

CANADA'S MOST SUCCESSFUL SUMMER OLYMPIC SPORT?

If you are interested please contact calrow@telusplanet.net

PRESENTED BY THE DEPARTMENT OF BIOMEDICAL ENGINEERING
IN FULFILMENT OF HIS PHD REQUIREMENTS

CHARLIE HSU



DISSERTATION SEMINAR

MECHANISTIC STUDIES ON THE UPTAKE AND
INTRACELLULAR TRAFFICKING OF LIPID-MODIFIED
CATIONIC POLYMERS FOR GENE DELIVERY IN PRIMARY CELLS

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OBOROWSKY DEGNER SEMINAR HALL // 1-040 LI KA SHING CENTRE



Represent Team Alberta at the 
2009 Canada Summer Games

Attention all athletes born in 1989 or later,
we are seeking highly motivated athletes with a proven sport
background interested in starting rowing, with the ultimate
goal of representing Alberta in August 2009 at the CSG in P.E.I.

For more information please visit <http://www.erc.edmonton.ab.ca>

Brought to you by the Alberta Rowing Association
in partnership with the Edmonton Rowing Club
and the University of Alberta Rowing Team



PROMOTION POSTERS

Another poster I did for the Alberta Rowing Association's Podium Project.

THESIS SEMINAR

I designed a poster for my thesis defence public seminar, but the department insisted on using their own Microsoft 3D text poster instead.

CANADA SUMMER GAMES

Another poster for the Alberta Rowing Association, for the 2009 Canada Summer Games.

Welcome!

To the Rancourt Lab

**Professor, Oncology, Biochemistry and Molecular Biology, Medical Genetics,
University of Calgary**

The Rancourt lab studies mouse and human preimplantation embryos. We have extensive experience with the derivation, expansion, differentiation and genetic manipulation of mouse and human embryonic stem cells and have begun to generate specific tissues for regenerative medicine applications. [Read More](#)

About .

Dr. Derrick Rancourt is a professor in the Departments of Oncology, Biochemistry & Molecular Biology, and Medical Genetics at the University of Calgary.

He is currently the Director of the ESTM Facility, a member of the Southern Alberta Cancer Research Institute, Deputy Director of the McCaig Institute for Bone & Joint Health, and Associate Scientific Staff Member of the Tom Baker Cancer Centre.

[→ More on People](#)

Research .

The Rancourt lab research program revolves around the derivation, expansion, differentiation and genetic manipulation of mouse and human pluripotent stem cells (PSCs), including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells.

Recently, we have discovered that fluid shear stress in SSBs induces pluripotency and significantly increases the efficiency of generating iPS cells.

[→ More on Research](#)

Opportunities .

The Rancourt lab is committed to providing opportunities for research trainees to further their scientific careers. Prospective lab members are evaluated based on the suitability of the applicants' background to current research goals and research projects.

Ideal candidates should have backgrounds in molecular biology and genetics. However, interested individuals from all disciplines are encouraged to apply.

[→ See Current Opportunities](#)

RANCOURT GROUP LAB WEBSITE

2013-Present

I re-did the website for my postdoctoral research supervisor. I used a template to save time on hard coding everything then customized the layout and graphics afterwards.

RESPONSIVE CONTENT

RANCOURT LAB

DYNAMIC VIEWING

The HTML/CSS template has built in responsive content functionality, which means viewing is optimized for both web and mobile devices



Research

People

Facility

News

Opportunities

Research Areas

Expansion and Derivation of pluripotent stem cells in stirred suspension bioreactor

Regenerative Medicine | Bioprocesses | Cell Culture

We are developing methods for expanding mouse and human pluripotent stem cells (i.e. embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)) as aggregates in stirred suspension bioreactors. Bioreactors offer numerous advantages over conventional static culture systems, which lack control and have low cell yields. Bioreactors can be controlled; physiological levels of key parameters can be maintained, and large amounts of cells/tissue can be grown in a short time. Human intervention is minimized, so there is little batch variability. This makes bioreactors an essential step in the application of stem cells. Having demonstrated that the quality of bioreactor expanded cells was equal if not superior to conventional static expansion methods we next explored our ability to differentiate mouse ESCs into different tissue types in the bioreactor. In sharp contrast to static culture, where cell differentiation occurred efficiently, we observed that many cells remained pluripotent in the suspension culture environment and causing them to be refractory to tissue differentiation.

Using the mouse model system, we recently demonstrated that in the bioreactor iPSCs form **1000-fold more efficiently, and in half-time compared to static culture**. The method is so efficient that we have begun to remove genes from the reprogramming process. Our current research is investigating how fluid shear promotes cellular reprogramming via mechanotransduction.

→ Latest Publications

Featured Publications

- Expansion and long-term maintenance of induced pluripotent stem cells in stirred suspension bioreactors.
- Derivation of iPSCs in stirred suspension bioreactors.
- Suspension bioreactor expansion of undifferentiated human embryonic stem cells.
- Reduced differentiation efficiency of murine embryonic stem cells in stirred suspension bioreactors.
- Embryonic stem cells remain highly pluripotent following long term expansion as aggregates in suspension bioreactors.
- Expansion of undifferentiated murine embryonic stem cells as aggregates in suspension culture bioreactors.

Osteoblast, Chondrocyte and Cardiomyocytes Differentiation

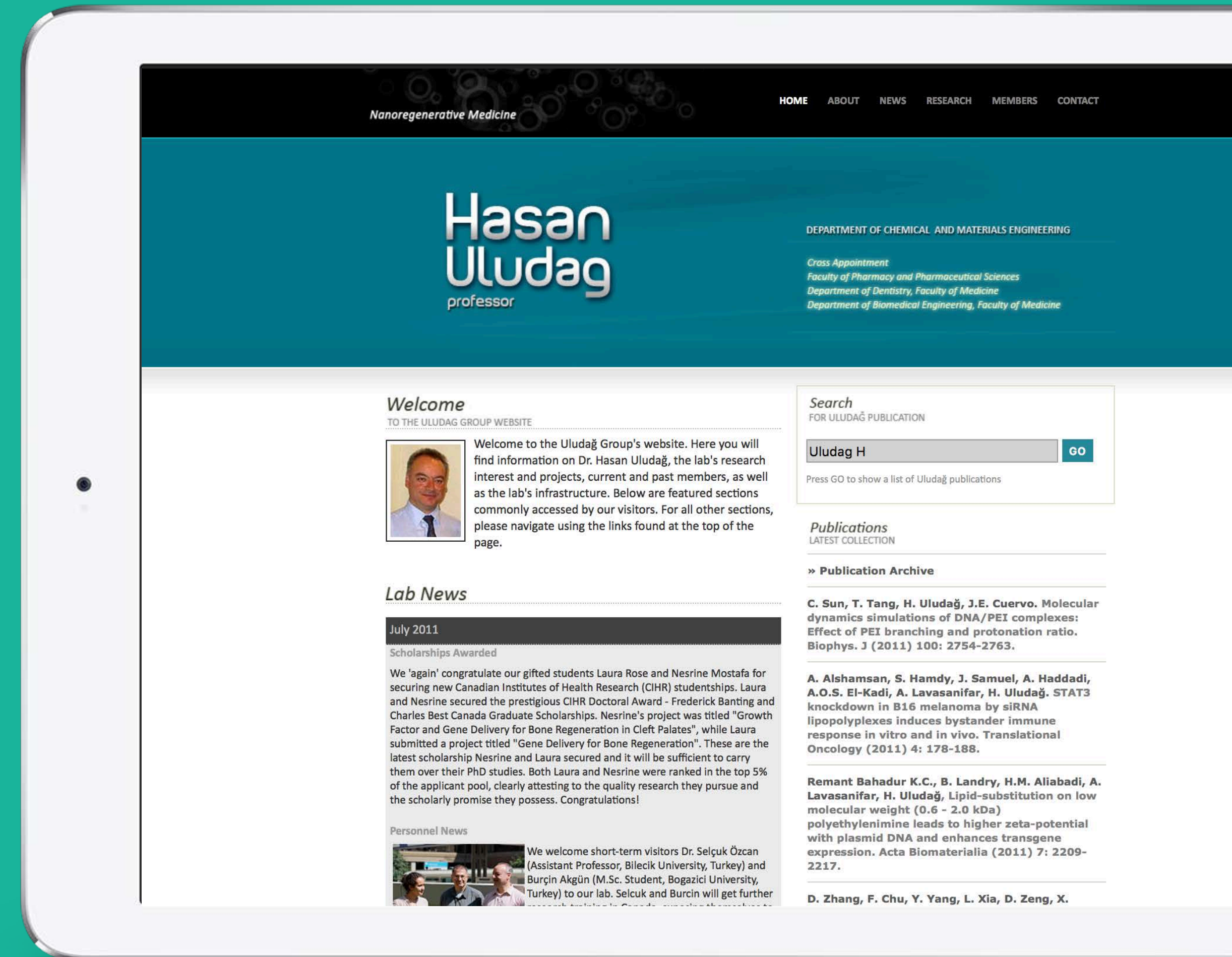
Tissue Engineering | Differentiation | Cell-based therapy

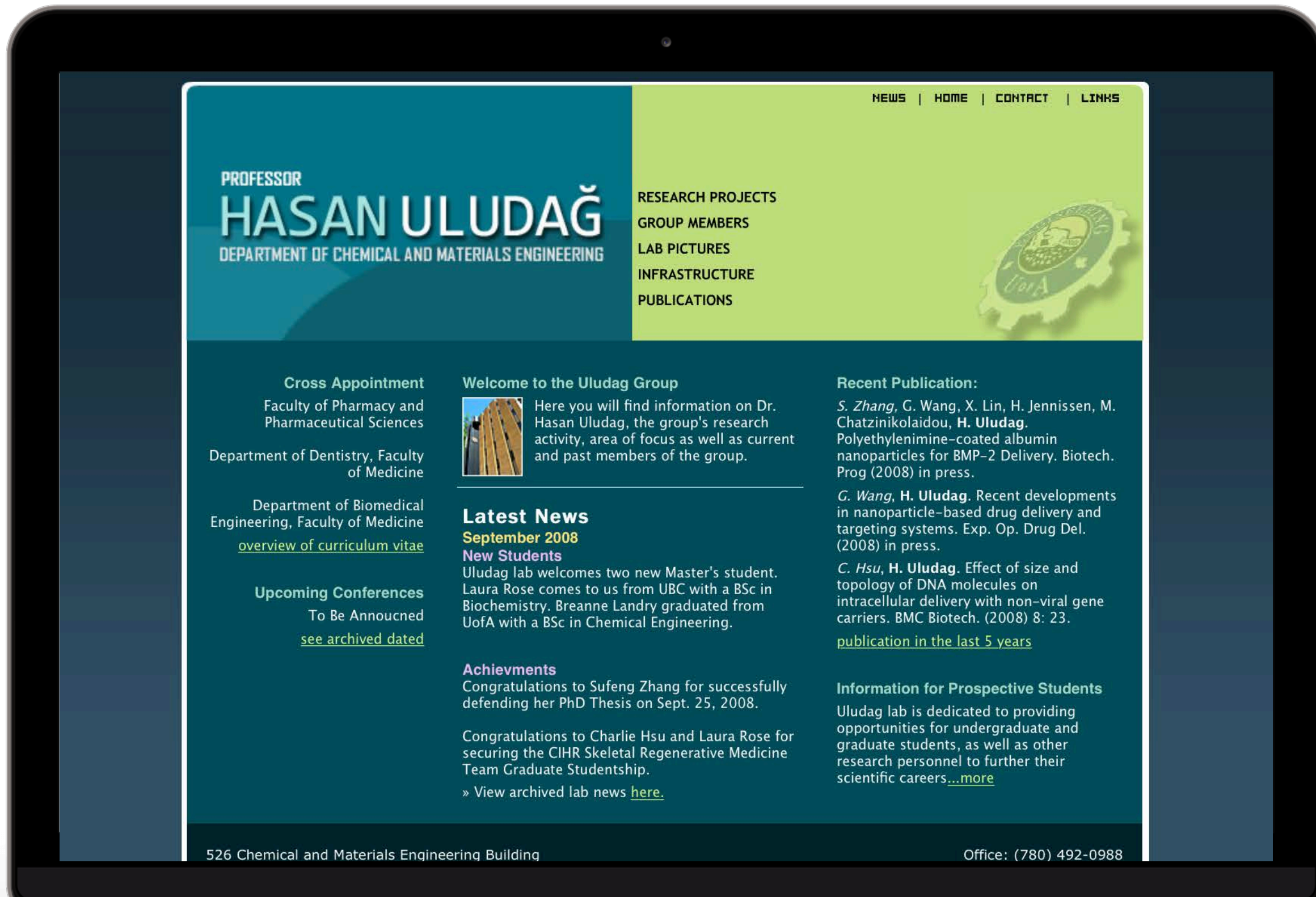
We are developing new methods for differentiating mouse and human pluripotent stem cells (PSCs) into osteoblasts, chondrocytes and cardiomyocytes and transplanting them into animal injury models. When cells are affixed to the bottom of micro-drop cultures and treated directly with differentiation factors, some cells undergo apoptosis while others form small chondrocyte or osteoblast fated aggregates in suspension. We have also developed methods to differentiate ESCs within collagen gels. These constructs are sufficient to induce osteoblast formation in the absence of any growth factors: ESCs are simply removed from pluripotency maintenance factors, suspended in collagen gels and cultured for 15 days whereupon they form osteoblasts or chondrocytes *in vitro*.

ULUDAG GROUP LAB WEBSITE

2009-2011

I also did the lab website for my doctoral thesis supervisor. This is from 2009-2011, pre-responsive content. I start migrating towards a Web 2.0 interface at this point. But this is still coding HTML/CSS by hand, which is very time consuming.





ULUDAG GROUP WEBSITE

2006-2009

The first version of the lab website that I did back in 2006. There was no advanced CSS functionalist, so limited to Flash-based menu with simple animation



Welcome to UofA Rowing



About Us

The mission of the UofA rowing team is to provide the opportunity for University of Alberta student-athletes to participate, train and compete in the sport of rowing, at all levels, from novice to high-performance, during the scholastic year. The UART is a non-profit organization run by a group of volunteers

Programs Offered

We are open to athletes and would-be athletes of all skill level and fitness. The UART offers tiered programs ranging from Learn-to-Rows to elite competition at the national level. We see our members as student before athlete, and understand the demand of school work. Thus we are committed to providing a learning environment suitable for the advancement of both your education and your aspiration to perform in the sport. For details on the various programs offered, check out the "Programs" section.

Membership

We understand that our members are students living on limited budget. That's why we are committed to bringing the cost to as low as possible. Through our fundraising effort, we are able to bring the total cost to half.

Bulletin Express

Learn-to-Row Review

Never rowed before? Want to try out rowing? Our fall Learn-to-Row is a comprehensive activity-based program designed for those with little or no prior experience and introduce them to the sport of rowing. The program will teach basics such as rowing biomechanics, proper rowing technique, as well as water safety. Instructions involve a combination of indoor exercise on the Concept2 indoor rowing ergometers, a indoor rowing tank, and of course, on the water, in a real rowing shell.

Calendar

Check out our page on the [Campus Recreation website](#) for the latest events and upcoming regattas

Find Us!

We are on Facebook! Look us up by searching "University of Alberta Rowing Team". Join the discussion, get the latest News Feed from the team, get involved, and contact the executives directly.



For all other general inquiries, email us at rowing@ualberta.ca.

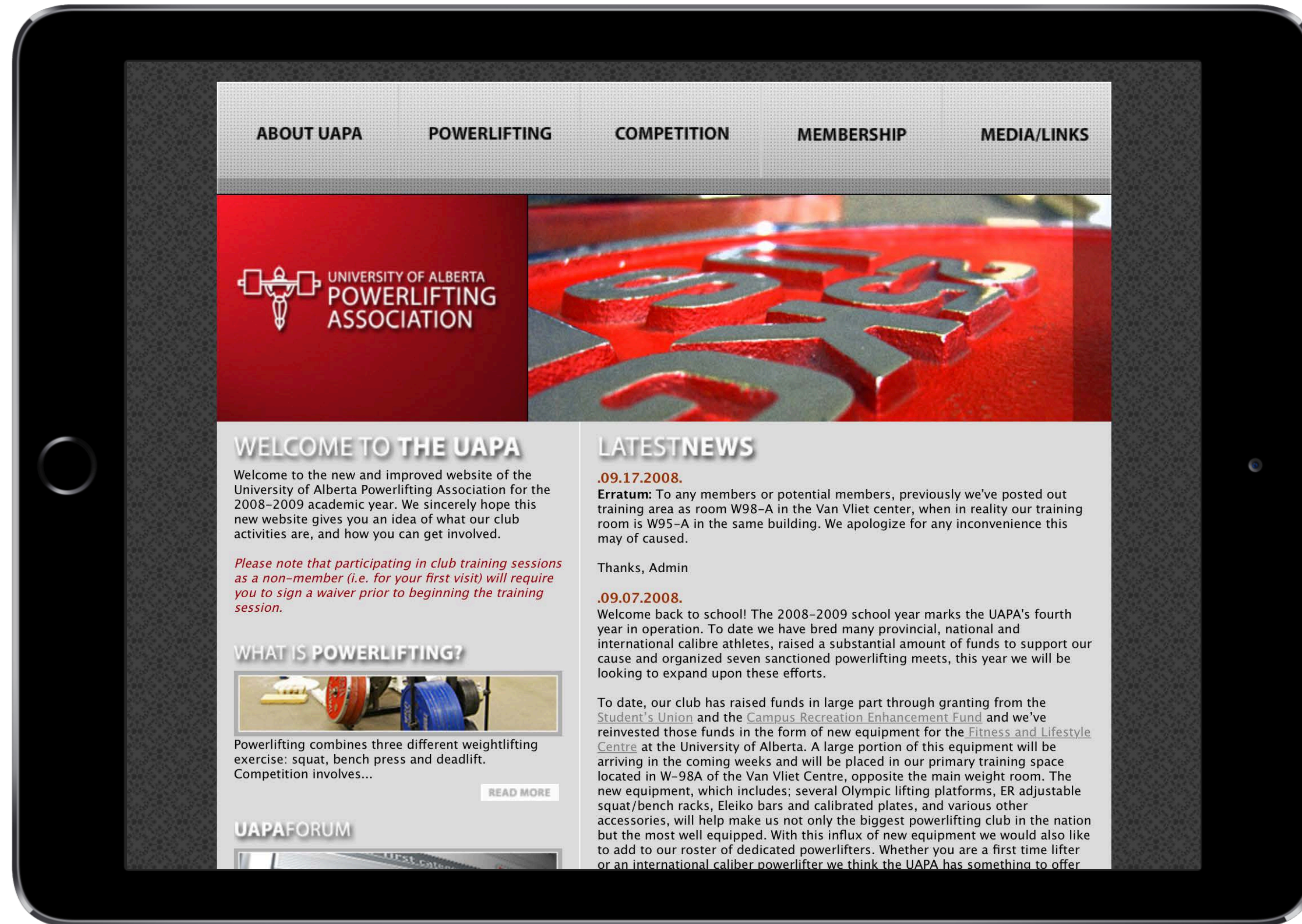
WEB DESIGN

UNIVERSITY ROWING

2006-2008

I was with the university rowing club from 2004-2010. This is the third website I did for the University of Alberta Rowing Team before migrating to a content management system.

The other two were done between 2004-2006, which did not have CSS or Web 2.0 features and were purely just HTML hardcoded.



POWERLIFTING CLUB

2007-2009

I was also part of the University of Alberta Powerlifting Association from 2007-2012. This was the first website I did for them before they went under the auspices of Alberta Powerlifting Union and adopted Facebook Page exclusively.

EDMONTON ROWING CLUB

I was with the Edmonton Rowing Club from 2004-2013. I did a number of websites for them. This is the longest running one from 2005-2008 before I implemented a Drupal-based content management system with a customer relation database

