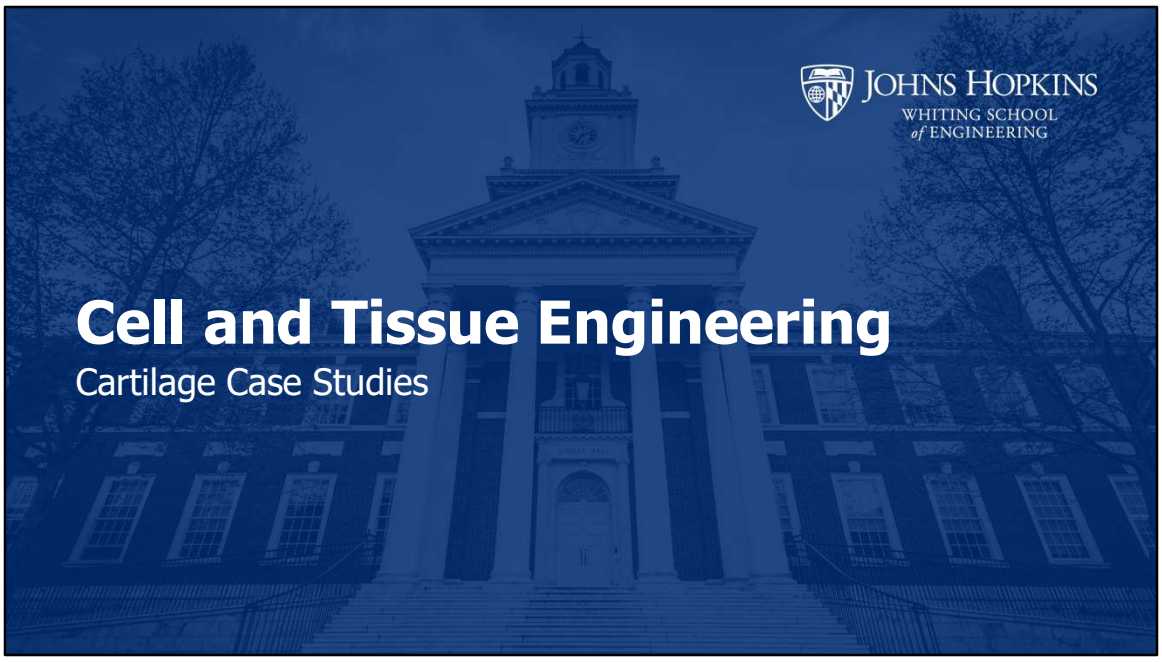
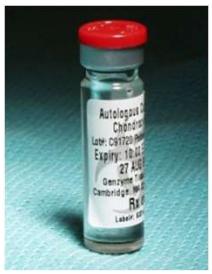


Welcome to Cell and Tissue Engineering. This is Ethan Nyberg and in this module, we are discussing case studies of tissue engineering products.

Case Studies in Tissue Engineering

Carticel (autologous cultured chondrocytes)



Epicel (cultured epidermal autografts)

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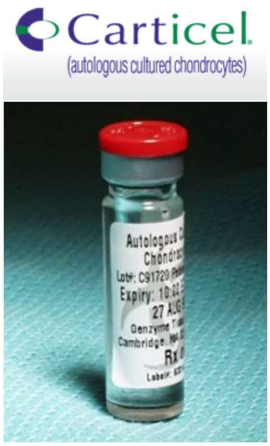
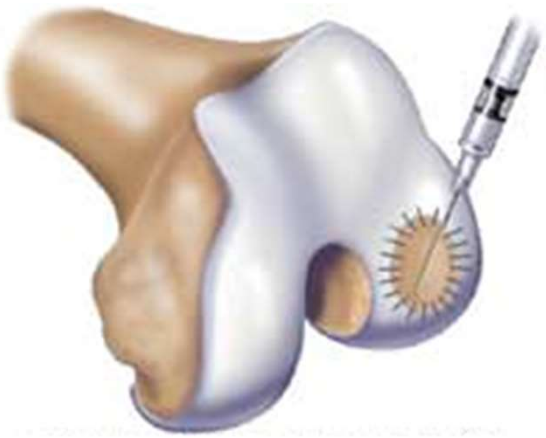
MACI, Smith & Nephew; GEM

Today we're going to cover three areas of tissue engineering – cartilage, skin and nerve.

Here are some products that we'll be talking about:
Carticel – autologous chondrocytes used for cartilage regeneration
Epicel – cultured autologous keratinocytes for skin regeneration
And **neurotube,** a nerve guide made of absorbable woven polyglycolic acid mesh desiged for nerve repairs less than 3cm.

Carticel: Tissue Engineered Cartilage





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Carticel.com

Today, let's begin by talking about **cartilage**.

One of the first products to hit the market from tissue engineers was in this category – Carticel is *simply* cultured autologous chondrocytes that are used to help repair cartilage defects in the knee. It was the only FDA approved treatment to use your own cells to treated damaged cartilage in your knee.

This solutions is indicated for the repair of symptomatic cartilage defects caused by releate trauma specifically in patients that have had inadequate responses to prior arthroscopic or other surgical repair techniques. It is not indicated for damaged associated with general osteoarthritis.

Autologous chondrocyte implantation (ACI) is a form of tissue engineering that creates a graft from an individual's own cartilage cells to repair defects in articular cartilage. The procedure involves surgical removal of a small piece of articular cartilage, harvesting of cells from the cartilage, growth of these cells in a specialized laboratory, and implanting the cultured cells over the cartilage lesion, with the goal of restoring resilient, durable cartilage at the site of injury.

The first-generation product, Carticel, is being phased out, but we will walk through how it worked in the following slides. Methods to improve the first-generation autologous chondrocyte implantation procedure include the use of a scaffold or matrix. There is currently one FDA approved matrix-induced autologous chondrocyte implantation product. In 2016, MACI® (matrix-induced autologous chondrocyte implantation [ACI]; Vericel Corporation) received FDA approval for the repair of symptomatic, full-thickness cartilage defects of the knee in adults. MACI is composed of biocompatible carbohydrates, protein polymers, or synthetics and is supplied in a sheet cut to size and fixed with fibrin glue. This procedure is considered technically easier and less time-consuming than the first-generation technique, which required suturing of a periosteal or collagen patch and injection of chondrocytes under the patch.

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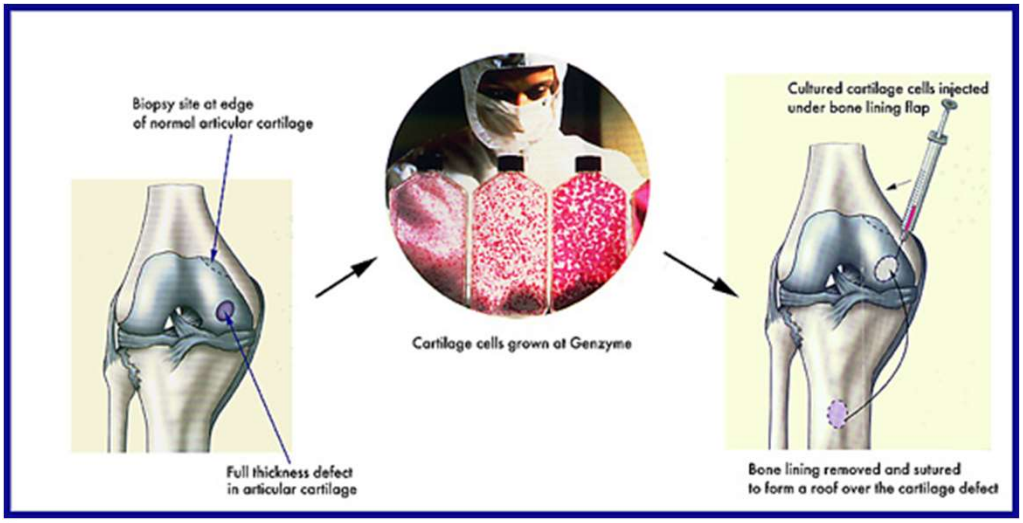
https://www.bcbst.com/mpmanual/!SSL!/WebHelp/Autologous_Chondrocyte_Implantation.htm

Carticel: Procedure



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Carticel.com

The procedure works like this:

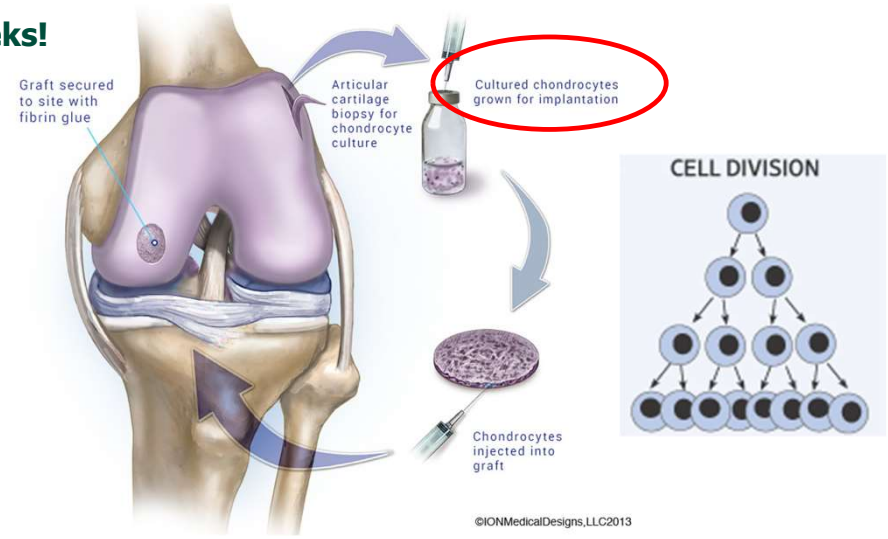
First a pea-sized biopsy is taken from healthy knee cartilage. The chondrocytes from this tissue are exanded *ex vivo* in the lab.

When sufficient cells are obtained, they are shipped back to the hospital.

A surgeon goes in and removes the damaged cartilage, implants the chondrocytes, and covers contains them with a mesh patch made of periosteum which is sutured to the surrounding healthy cartilage. In MACI, the periosteum flap is replaced with a porcine collagen membrane that is fixed in place using fibrin glue.

Carticel: Cell Expansion

5-6 weeks!



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The surgeons does both the **excision** and the **implantation** – so the real job of the tissue engineer in these products are during the **expansion** of the cells.

The tissue engineer needs to work with the limited number of cells that they get from the biopsy and then use culture techniques to **amplify** this population in an acceptable **window of time**. For this product the amplification phase runs approximately 5-6 weeks.

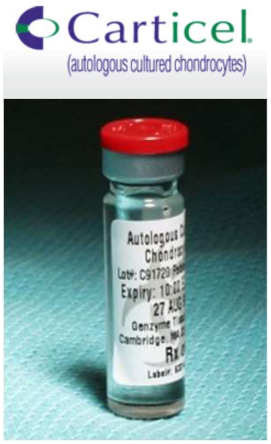
Carticel: Cell Expansion by the Numbers

Median dose: 1.6million cells per cm^2

Packaged at ~12million per vial

Genzyme provides

- 1 vial for defects $<7cm^2$
- 2 vials for defects $>7cm^2$ and $<14cm^2$
- no testing for infectious diseases



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The tissue engineers here are at the company Genzyme (now Vericel).

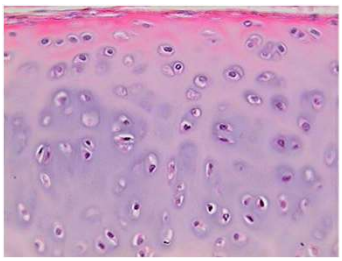
They take the pea-sized biopsy and grow it as needed for the anticipated defect area. In their clinical trial the median dose **was 1.6 million** cells per cm2. Genzyme packages the samples at 12 million cells per vial and provides 1-2 vials to the surgeon for placement. Their product is **not** tested for infectous diseases and is **limited for autologous** transplants only.

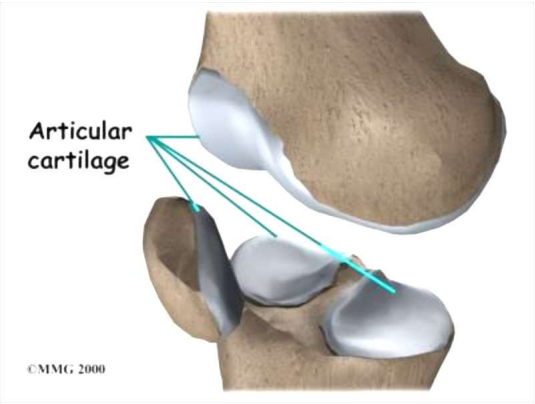
They also require that surgeons take their **Biosurgery Training Program** which includes a surgical training prior to implantation procedures.

So, although the company Genzyme is acting as the **tissue engineer**, they are also regulating exactly how the **surgery** is performed, and how their **product** is used.

What Does Carticel Do?

- Forms new hyaline cartilage
- Repairs the injury site
- Reduces pain
- Improves joint movement and function
- Does not damage the underlying bone





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wikispaces.com

We know **what** carticel is, and **how** it is used, but what exactly **does it do** for the patient.

Articular cartilage is hyaline cartilage that is on the surface of bone. You can see the dark purple chondrocytes sitting in the lacunae indicative of this cartilage structure in the histology image on the upper right.

The implantation provides a first repair to the injury site – that is it **fills the void** left after the damaged cartilage is removed. This improves the function of the joint and reduces pain by providing a smoothly articulating surface.

Second, when newly expanded chondrocytes are placed put in a newly cleared injury site, they begin to **reform the bone and cartilage**. This engineered solution replaces damaged hyaline cartilage – in this case articular cartilage – with a **chemically** and **structurally** similar material.

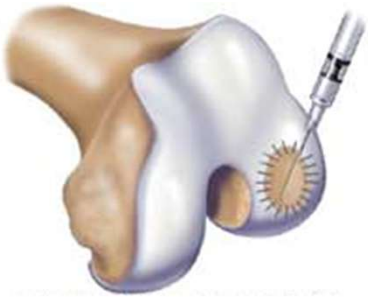
It is important to mention that these new chondrocytes do not damage the existing bone structure beneath the surgical site. When we fix something in tissue engineering, we do not aim to do so at the cost of something else – thus why the

second phase of the product uses a porcine collagen membrane instead of a periosteum graft.

Carticel: Adverse Events

Study of the Treatment of Articular Cartilage Trial (STAR)

Adverse Event	% of 154 patients
Arthrofibrosis/Joint Adhesions	16%
Graft Overgrowth	15%
Chrondromalacia or Chondrosis	12%
Cartilage Injury	11%
Graft Complication	10%
Meniscal Lesion	8%



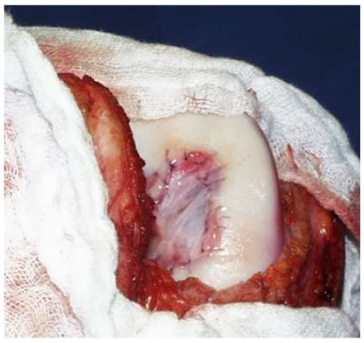


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Table adapted from fda.gov

This solution performed **very** well in clinical trials – however there were cases where things did not work out as planned. In the STAR trial – the study of the treatment of articular cartilage -- which was completed in 2009, 154 patients were tested at 29 different surgical centers. Adverse Events from the clinical trial are outlined for you in this table. I'm showing you these to emphasize that no therapy is **perfect**.

However, the clinical trial resulted in **serious adverse** effects in up to 16% of the 154 patients studied. The most prevalent effects being **joint adhesions** and **graft overgrowth**. Cartilage injuries include **new ones** not related to the study.

The most clinically significant intervention after implantation was removal of the graft due to **partial or total delamination** of the periosteal flap. IN the clinical trial this occurred in 4 of 154 patients – You can understand from our earlier lectures how the mechanical mismatch at the joining of the graft to the cartilage can lead to delamination. Arthofibrosis also could stem from a mechanical mismatch leading to an inflammatory fibrotic response. Finally, we have covered how important it is to provide adequate numbers of cells for the purpose – in this case you can see how overgrowth could occur from our models of cell growth.

This therapy was approved and **widely** used in the US and Europe, where they were approved (via BLA) in 1997 and had reached 6000 patients by 2001. Eventually, they scaled back to only the US market because of the regulatory hurdles of maintaining a cell processing facility in Europe. In Q3 of 2017, marketing of Carticel ceased and the company now markets a 3rd generation autologous cartilage repair product - MACI. You'll be thinking more about this product in your homework this week, so we'll stop here.

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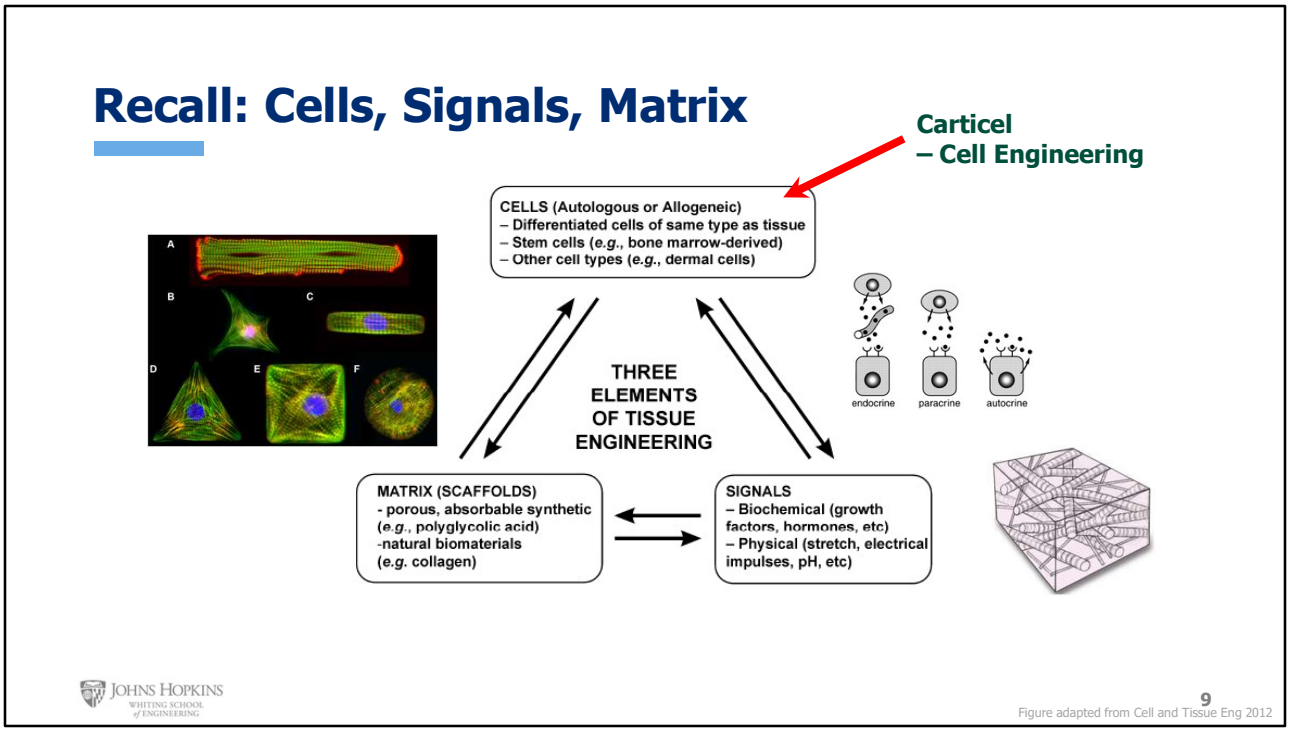
https://www.annualreports.com/HostedData/AnnualReports/PDF/NASDAQ_VCEL_2020.pdf



There are a number of other solution methods for repairing cartilage – recall from our first lecture this semester, the tissue engineering paradigm. This trifecta includes cells, signals and matrix.

Not all solutions use every component. In fact, the **carticel** solution really *only* used cells – and *perhaps* signals if you include the culture conditions used to expand the cells. It's successor, MACI, used the same cells in combination with a collagen matrix scaffold – two components of this triangle.

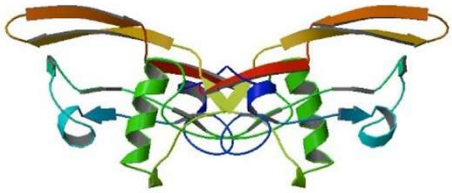
Let's take a moment now to consider solutions that **do not use cells** – that rely on the other two vertices on the bottom of this triangle.

Cell-free Cartilage Engineering

- **Proliferation** of surrounding chondrocytes
- **Differentiation** of local adult stem cells
- **Transdifferentiation** of local plastic cell type

Signals – Growth Factors

- Transforming Growth Factor beta: TGF-β
- 25kDa homodimer



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Re'em et al 2012

If you want to repair cartilage without supplying cells, then the solution needs to **stimulate the production** of new cells *in vivo.*

In cartilage repair, this production could be the **proliferation** of surrounding chondrocytes, the **differentiation** of local adult stem cells or the **transdifferentiation** of a local plastic cell type.

One factor that has been investigated in cell-free cartialge repair is transforming growth factor beta- or TGF-beta. You'll hear more about bone morphogenic proteins later in this series, these proteins are all members of the TGF-beta super family.

This molecule is needed not only for the **onset** but also the **maintenance** of chondrogenesis. This is a *secreted* protein that homodimersizes to forma 25kDa active molecule. You see that dimerized protein structure here on the bottom.

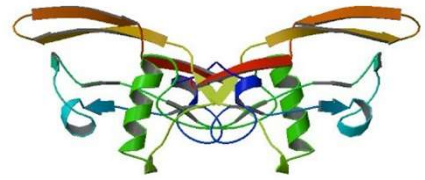
Biomaterial Scaffolds Release Growth Factors

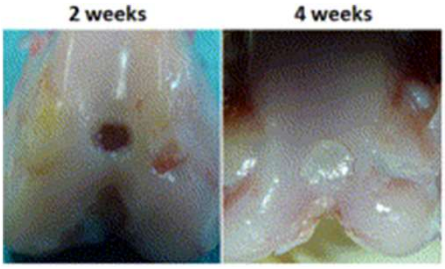
Growth Factors

- Transforming Growth Factor beta: TGF-β
- 25kDa homodimer

Scaffolds

- Hydrogels of polysaccharides, hyaluronic acid, alginate, fibrin, gelatin and collagen
- Biopolymeric gels
- Synthetic polymers
- Solid and hybrid scaffolds





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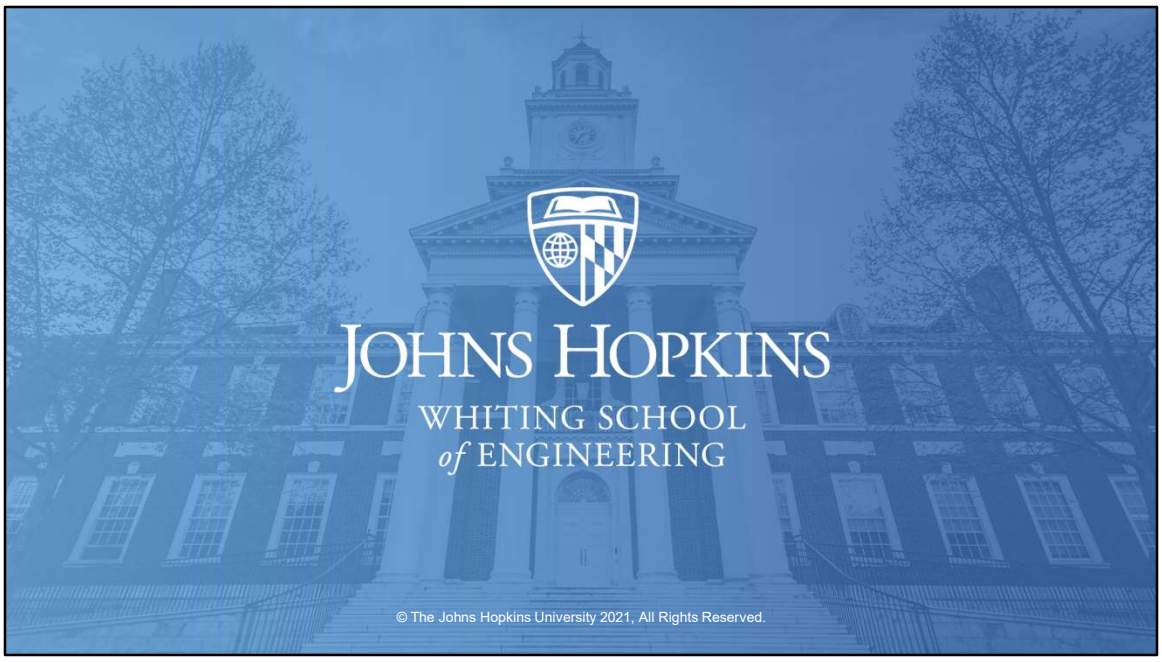
Re'em et al 2012

With this in mind, many scaffolds have been developed which control the **spatial** and **temporal** expression of TGF-beta. This has been investigated in **hydrogels** of various composition, synthetic **polymers** and **composite** materials which contail both solid and hydrogel components.

We know already from our module on biomaterials that these can each be tailored with specific **degradation** rates, **gelling** conditions, **pore sizes**, and **mechanical** properties.

In this image on the bottom right, you are looking at a rabbit model of cartilage treatment. You can see the **injury zone** is the dark red circle in the center at 2 weeks – that is the area of removed articular cartilage. A hydrogel **presenting *only* TGFbeta 1 and BMP4** was placed into the defect, and after 4 weeks, you can see the **start** of cartilage regeneration.

Histological analysis detected cartilage **proteoglycans** and **collagen II,** both classical markers of hyaline cartilage.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