

# **Burst Binate Patch**

**Research Brochure**



# Burst Binate Patch

**Protective barrier and wound healing for surgical and non-surgical procedures.**

The Burst Binate Patch is a dual-layered amniotic membrane commonly used as an adhesion barrier or wound care dressing.

**The Next Generation in Wound Healing**





## What is the Burst Binate Patch?

The Burst Binate Patch is a dual-layered amniotic membrane that serves as an ideal biologic barrier for wound care.

Our patch product consists of 100 percent amniotic tissue. It's manufactured through our proprietary Emergence Process to retain the tissue's inherent tensile strength, as well as a potent variety of naturally-occurring growth factors and cytokines.

Doctors rely on the patch's consistency, stability, and versatility for a range of procedures.

## How It's Used

The Burst Binate Patch is utilized as an adhesion barrier or wound care dressing in both surgical and non-surgical procedures.

The product is routinely used in orthopedic care, podiatry, plastic surgery, and general surgical applications.

## Our Research

We have a team of scientists conducting studies on the Burst Binate Patch in our onsite laboratory. Throughout this brochure, we share some of our internal translational research studies on the product. Much of this research was featured in our recently-published paper "Biochemical characterization of pure dehydrated binate amniotic membrane: role of cytokines in the spotlight" in the journal *Regenerative Medicine*. These studies demonstrate the power of the Burst Binate Patch in action.

# Histological Images of Burst Binate Patch

In order to promote a healing effect, the patch needs to retain its native structural complexity post-dehydration. After dehydration, we captured histological images of the Burst Binate Patch (Figure 1). These images show that the patch's structural integrity remained partially intact.

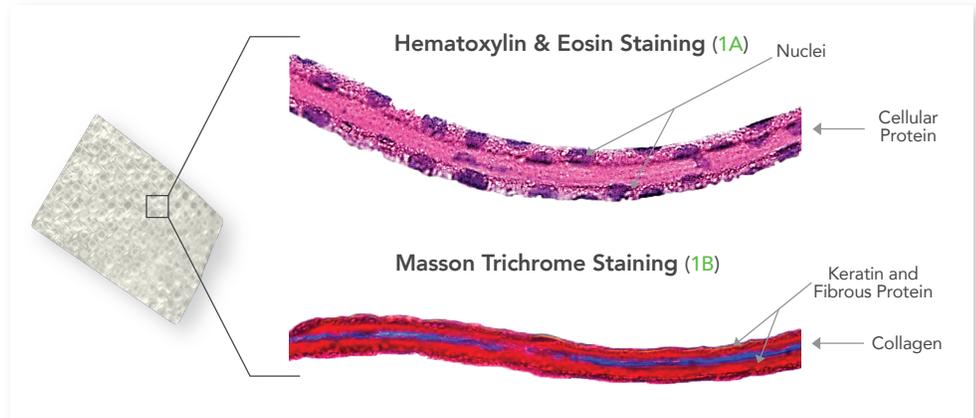


Figure 1

Burst Binate Patches (N=3 donors) were embedded in resin, cross-sectioned into 5  $\mu\text{m}$  thickness, and then stained with hematoxylin and eosin (Figure 1A) or trichrome (Figure 1B).

Hematoxylin (purple) stains the cell nuclei, and eosin (pink) stains the protein. For trichrome staining, blue stains represent collagen and red stains represent keratin and other fibrous proteins. Representative images are captured at 20 times magnification.

## Burst Binate Patch: A Storehouse of Cytokines and Growth Factors

Cytokines/Growth Factors	Mean Value (pg/mg)	Function
Granulocyte-Colony Stimulating Factor (G-CSF)	134.85 $\pm$ 85.33	Stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream
Vascular Endothelial Growth Factor (VEGF- $\alpha$ )	54.68 $\pm$ 9.20	Stimulates endothelial cell migration, proliferation, and survival; potent stimulator of angiogenesis
Epidermal Growth Factor (EGF)	50.11 $\pm$ 21.31	Acts as potent mitogenic factor that plays an important role in growth, proliferation, and differentiation
Stromal Cell-Derived Factor 1 (SDF-1 $\alpha$ )	11.97 $\pm$ 0.12	Plays a major role in cell trafficking and homing of CD34(+) stem cells that aid in regeneration
Interleukin 6 (IL-6)	3.88 $\pm$ 0.90	Responsible for stimulating acute phase protein synthesis and production of neutrophils in the bone marrow
Platelet-Derived Growth Factor-BB (PDGF-BB)	0.77 $\pm$ 0.16	Potent chemoattractant and activator of neutrophils and monocytes; increases the synthesis of phospholipids, cholesterol esters, glycogen, and prostoglandins; modulates LDL receptor binding
Interleukin 8 (IL-8)	0.75 $\pm$ 0.08	Chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes
Transforming Growth Factor $\alpha$ (TGF $\alpha$ )	0.44 $\pm$ 0.26	Activates a signaling pathway for cell proliferation, differentiation, and development
Interleukin 10 (IL-10)	0.21 $\pm$ 0.02	Anti-inflammatory cytokine with pleiotropic immunoregulatory role

N=6 donors. The concentrations for each factor were normalized to the dry weight of the patch. Data is represented as mean values  $\pm$  SEM.

Figure 2

Growth factors, chemokines, and cytokines are important modulators of inflammation, angiogenesis, and wound healing. To quantify these factors in the Burst Binate Patch, we homogenized the patch in phosphate buffer saline and analyzed it with the MAGPIX<sup>TM</sup> multiplexing system. As Figure 2 shows, the Burst Binate Patch contains a rich concentration of signaling molecules that promote a healing effect.

# Burst Binate Patch Stimulates Cell Proliferation *in vitro*

The proliferative phase involves the formation of new tissue, a critical part of effective wound healing. We wanted to determine the effects of the Burst Binate Patch on cell proliferation *in vitro*. For the assay, we treated bone marrow stromal cells (BM-SC) and mesenchymal stem cells (MSC) either with the basal media devoid of any growth factors or with basal media supplemented with the homogenized extract of the binate patch (2 mg/mL) from 3-5 different donors (Figure 3).

Relative proliferation was measured by fluorescence quantification of the total cellular DNA (CyQUANT assay) and is represented as the fold change of growth observed in basal media after 48 hours (Figure 3.2 and Figure 3.3). Ultimately, cells treated with the patch saw a dramatic proliferation compared to that of the control. The Burst Binate Patch contributes to the wound healing response through proliferation of bone marrow stromal and mesenchymal stem cells.

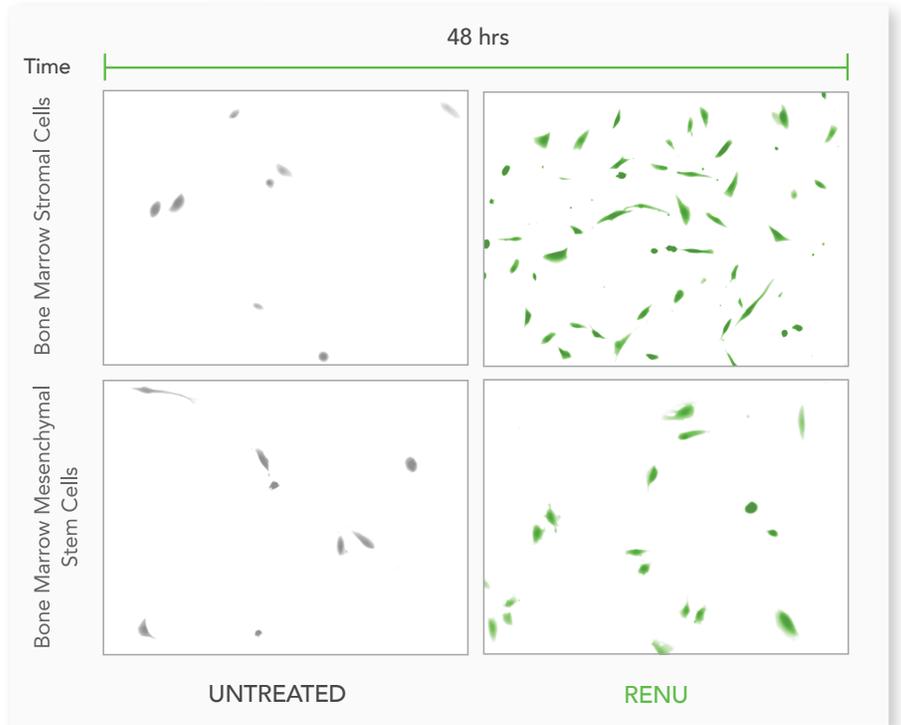


Figure 3.1

Cells growing in completed media (10% FBS) served as positive experimental control. Live cells were also stained with the cell permeant Calcein AM fluorescent dye and imaged at 40 times magnification to visualize the number of viable cells in randomly chosen fields. Experiments were repeated three times in triplicates and  $p < 0.05$  (\*) was considered statistically significant.

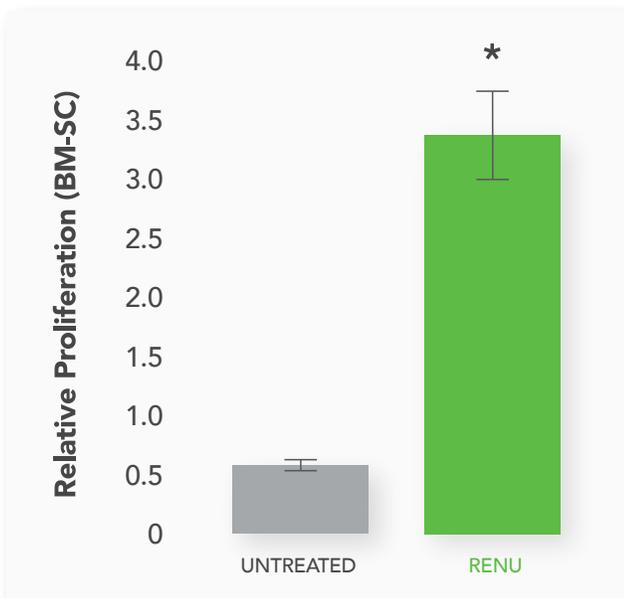


Figure 3.2

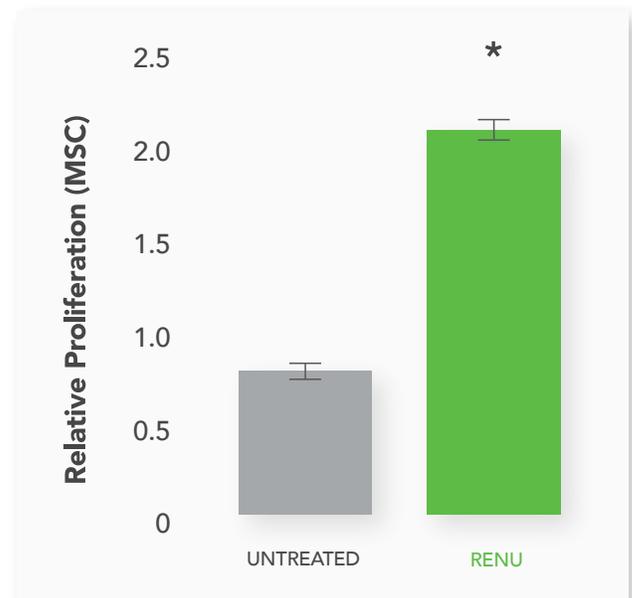
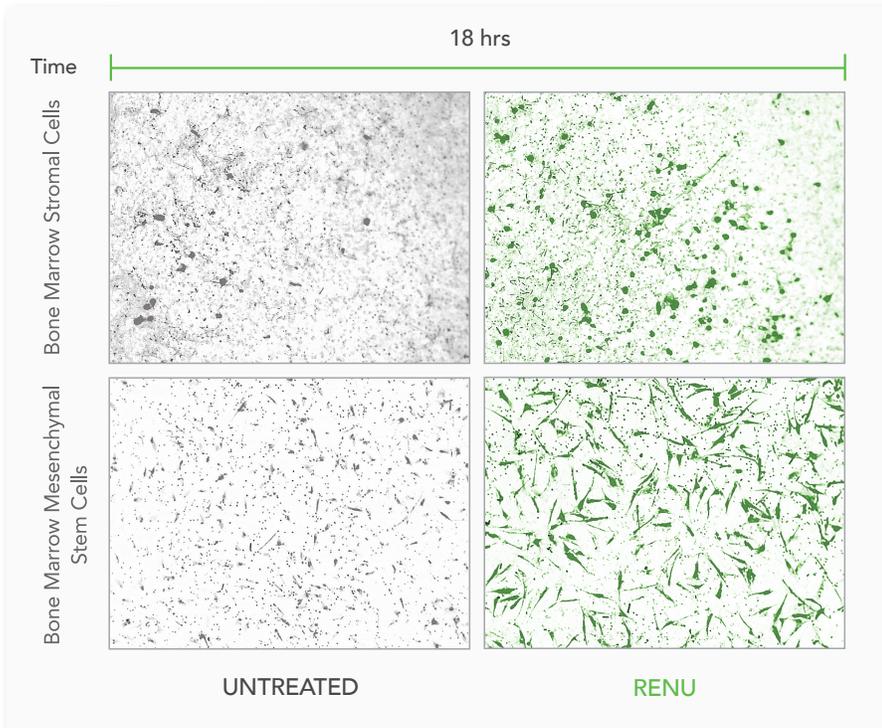


Figure 3.3

# Burst Binate Patch Stimulates Cell Migration *in vitro*



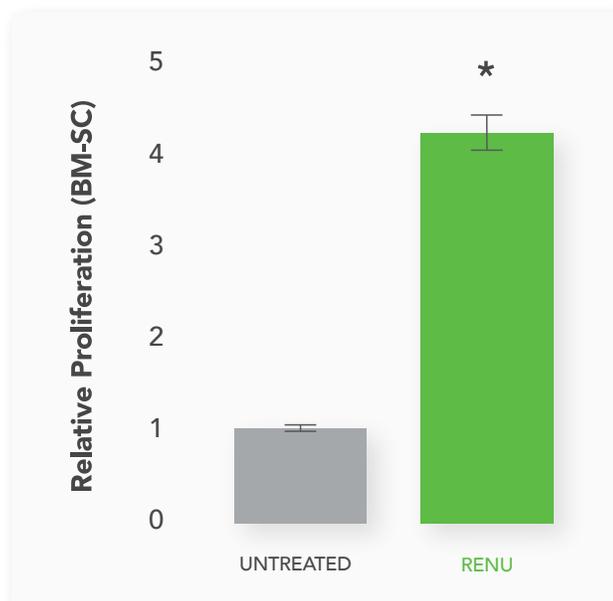
**Figure 4.1**

Quantification of the images was performed by ImageJ software from NIH. Experiments were repeated three times in triplicates and  $p < 0.05$  (\*) was considered statistically significant.

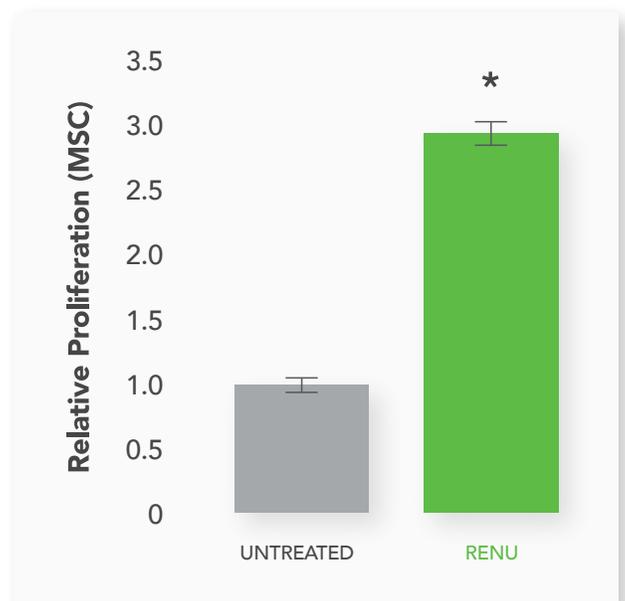
Recruitment of stromal and mesenchymal stem cells facilitates wound healing. Through the modified Boyden chamber assay, we analyzed the migration of these key cells in response to the Burst Binate Patch *in vitro*.

Cells were cultured overnight in basal media without any growth factors, and then replated in the upper layer of a cell culture insert with permeable membrane and basal media alone, or supplemented with homogenates of the Binate Patch (2 mg/mL) placed below the cell permeable membrane.

Following an incubation period of 18 hours, the cells that migrated through the membrane were stained and counted (Figure 4.1). We saw a relative migration of 3-4 times as many cells when the culture was treated with the patch (Figure 4.2 and Figure 4.3), compared to that of the control. The Burst Binate Patch significantly increases cell migration for wound healing.



**Figure 4.2**



**Figure 4.3**

NOTE: The Renu-treated cells in Figure 4.1 were stained with crystal violet and colored green for representation.

# Burst Binate Patch Stimulates Cytokine Production

Wound healing depends on the secretion of cytokines and growth factors by bone marrow stromal cells (Figure 5.1) and bone marrow mesenchymal stem cells (Figure 5.2). We treated these cells with the Binate Patch homogenate (2 mg/mL) for 48 hours.

The net concentrations of those factors revealed a notable boost in cytokine production by the Burst Binate Patch compared to the basal media.

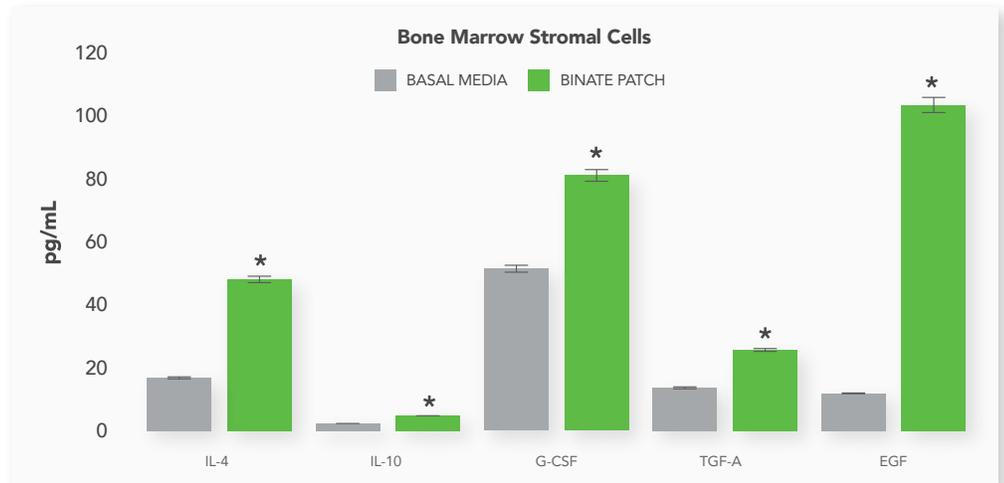


Figure 5.1

Total cellular secretome concentration was determined by MAGPIX multiplex analyzer and reported as picograms per milliliter, after accounting for the net concentrations of those factors in the initial homogenate and normalizing to the cell proliferative effect over 48 hours. Experiments were repeated three times in duplicates and  $p < 0.05$  (\*) was considered statistically significant.

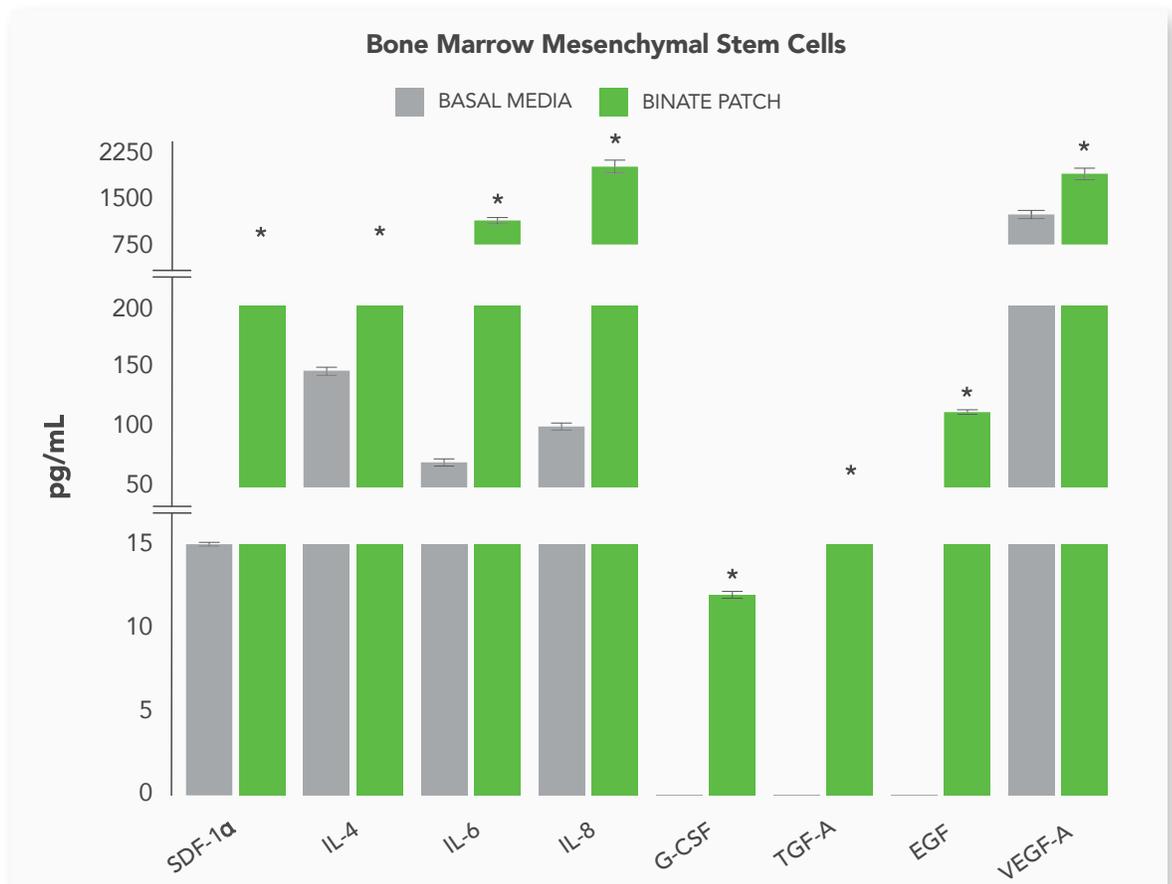


Figure 5.2

# Burst Binate Patch Demonstrates Thermal Stability for Biological Activity

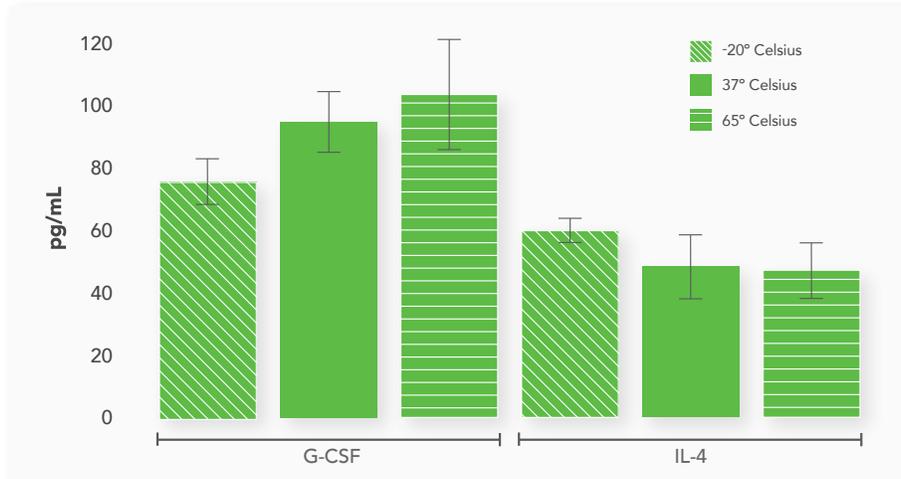


Figure 6.1

The biological activity of the Burst Binate Patch at a wide range of temperatures has a direct impact on the storage, stability, and shelf life of the product. We quantified the effect of thermal stability of bioactivity of the Burst Binate Patch on cytokine content (Figure 6.1) and the cellular proliferation rate (Figure 6.2 and 6.3) of bone marrow stromal and mesenchymal stem cells with the MAGPIX multiplexing system and CyQUANT assay, respectively.

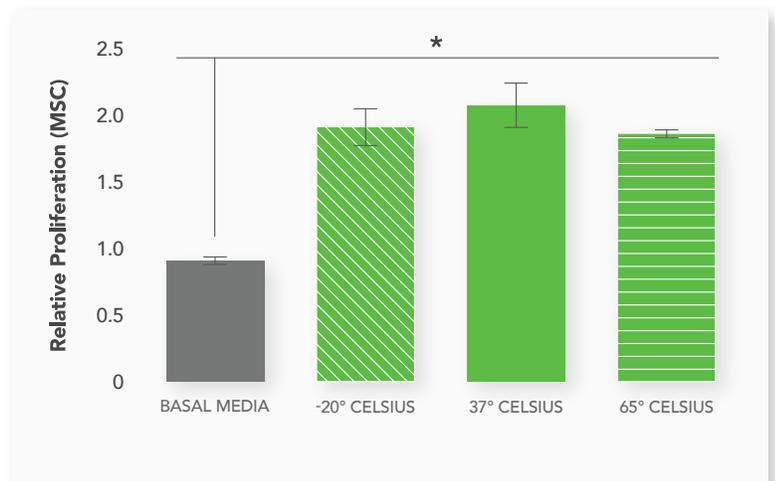


Figure 6.2

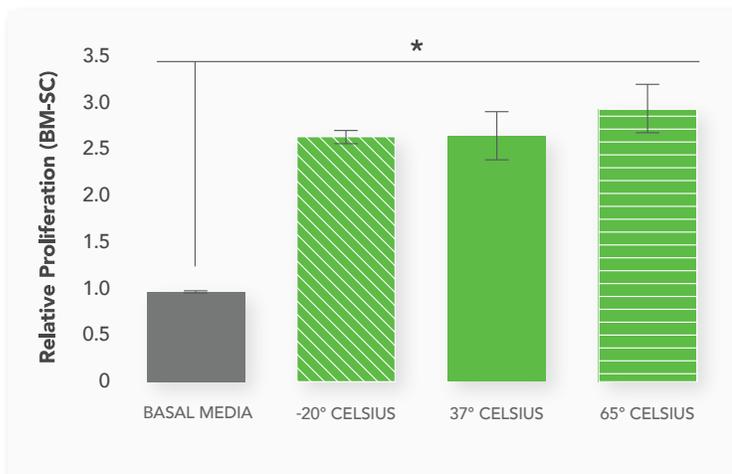


Figure 6.3

The cells were cultured for 48 to 72 hours in the absence or presence of 2 mg/mL homogenized extract of the Burst Binate Patch, which was conditioned at the indicated temperatures for 48 hours. For cell proliferation, data is presented as fold change  $\pm$  standard deviation relative to basal media. Experiments are performed in triplicates with N=3 donors.



**At Burst Biologics, our mission is to harness the power of biology.**

This mission is encompassed in the Emergence Process, our unique processing philosophy and methodology for the Burst Binate Patch.

### What is Emergence?

There is a natural phenomenon known as emergence describing how complex systems and entities possess properties that their individual components do not.

At Burst Biologics, we were inspired by this incredible phenomenon – so we brought it into the lab. Through the Emergence Process, Burst Biologics is redefining what's possible.

### Burst Binate Patch Embodies the Process

We developed the Emergence Process to create unique and transformative products in the wound care space. This exclusive methodology ensures that the Burst Binate Patch captures the philosophy of emergence, driving complex biological processes that have the potential to support wound healing and provide protection.



#### Consistency

Designed with minimum variability for reliable product quality.



#### Stability

Maintains product utility in a wide range of temperatures and applications.



#### Synergy

Combines research and manufacturing expertise for an amplified impact.



#### Protection

Produces powerful biomaterial barriers that can secure wounds and promote a healing effect.

## Who We Are

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Burst Biologics is a medical research and biologics company located in Boise, Idaho.

The company has established a sterling reputation in the field of regenerative medicine with its groundbreaking fluid allografts derived from umbilical cord blood, acellular allografts comprised of cancellous bone, and wound care products made from placental tissue.

Burst is committed to harnessing the power of biology to transform people's lives.



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