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Development of effective in-office autologous cell therapy for osteoarthritis

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Osteoarthritis (OA) is defined as the clinical and pathological outcome of a range of disorders that result in structural and functional failure of synovial joints[1] and is associated with progressive and irreversible destruction of articular cartilage that leads to chronic pain, joint instability, swelling, stiffness, and radiographic joint space narrowing[2]. OA is the leading cause of chronic disability among older adults and currently affects 300 million patients worldwide[3] (32.5 million people in the US alone)[4], representing the most common joint disorder worldwide and the most reported musculoskeletal disease[5] (Figure 1). The prevalenceof OA increases with aging, with 43% of patients being 65 or older;the development of OA is also associated with an increased risk of health conditions, including heart disease and diabetes[6–8].

The research proposed under this grant will complete the development of a clinical regenerative protocol based on the combination of autologous adipose-derived stromal cells (ADSC) and supporting viscosupplementation for the treatment of osteoarthritis (OA). The solution proposed by Jointechlabs (JTL) will disrupt the clinical practice by being the first therapeutic approach enabling cartilage restoration, overcoming current solutions which are primarily aimed at reducing pain and improving joint function. JTL will fill the gap in the use of regenerative autologous adipose-derived stromal Cells (ADSCs) in clinical practice, which has been so fair impaired by the lack of standardized clinical protocols for their isolation and poor survival after transplantation.

Challenges

Problem #1: There are no curative treatments available for OA. Acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics, serotonin-norepinephrine reuptake inhibitors (SNRIs), and intra-articular injections of corticosteroids and hyaluronic acid are used in the treatment of OA, depending on the severity and inflammatory state[9]. None of them can arrest the progressive degeneration of cartilage[10], and concerns about their gastrointestinal[11] and cardiovascular[12] side effects and the risk of opioid abuse[13] should greatly restrict their application. In case of severe joint degeneration, surgical procedures for implantation of artificial joint structures (arthroplasty) or total joint replacement (TJR) may be undertaken[14,15]. However, surgical interventions require long rehabilitation and expose patients to the risk of complications including infections[16], blood clotting, and nerve injury[17,18].

Problem #2: Available therapies have little-to-no effect on Health-related Quality of Life (HRQoL). The use of anti-inflammatory agents against OA has proven inconsistent in improving patients' HRQoL[19], and analgesics have no impact on HRQoL of OA patients as measured by SF-36 Physical Component Score (PCS) and Mental Component Score (MCS) through 1 year follow- up[20]. The injection of hyaluronic acid results in only 4% improvements in MCS at 24 weeks, and the benefit on HRQoL decreases after repeated administrations[21]. In patients waiting for total knee arthroplasty (TKA), PCS and MCS scores are well below the 25th percentile compared to age- and gender-matched normal values[22–24]; moreover, postoperative HRQoL improvements following TKA are not durable, with declines identified around 3 years post-surgery[25].

Problem #3: High economic burden of OA. The overall burden associated with OA in the US is estimated at $136.8 B (billion) annually[26], an amount that surpasses that of tobacco-related health effects, cancer, and diabetes. Direct medical costs reach $65 B annually, with indirect costs (i.e. lost earnings and absenteeism) of $10.3 B and annual all-cause per person direct costs of $11,502[26].

JTL is developing an innovative regenerative protocol for OA that could be easily implemented at POC(Point of Care). JTL approach to OA combines for the first time the regenerative healing properties of stem cells and the benefits of long-lasting viscosupplementation.

Value propositions

Value #1: JTL is offering the first curative, regenerative treatment for OA. The clinical protocol proposed by JTL will enable the regeneration of the cartilage degraded by OA, overcoming side effects and abuse risks associated with anti-inflammatory drugs and analgesics and the long rehabilitation required after arthroplasty procedures. The articular injection of autologous ADSCs isolated at the bedside and encapsulated in a protective hydrogel will support and accelerate the release of regenerative cellular factors to promote joint healing in OA.

Value #2: Improved patients' quality of life. The regenerative approach to restoring degraded cartilage willprevent the physical limitation, pain, functional limitations in daily-life activities, and social isolationassociated with OA progression. The long-term cartilage stimulation will outperform the short-term HRQoL improvements enabled by pharmacologic and surgical therapeutic strategies.

Value #3: Cost savings for patients and the healthcare system. JTL will propose its technology as a therapy protocol at a competitive price of $5,000. Hospitals and clinics will avoid the expensive protocols (exceeding $50,000)[27] to extract cells from the Stromal Vascular Fraction (SVF) and the average of $14,500 needed to perform total joint arthroplasty (TJA)[28], while OA patients will avoid expensive medical care (average patient-per-year costs of $11,200 for NSAIDs)[29] resulting in a total $36.1 B/year burden in out-of-pocket costs[30].

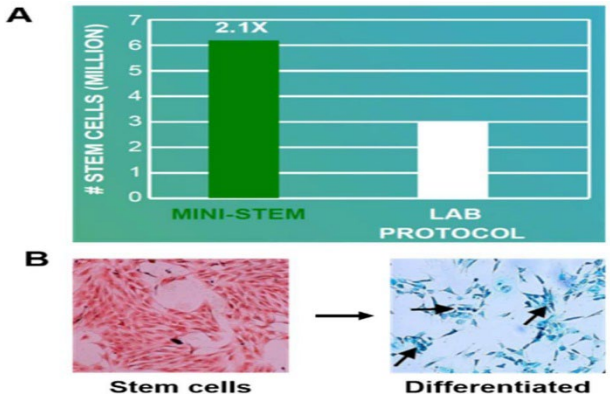
Impact of the project

The high prevalence of OA in the US results in 20.78 million ambulatory care visits and 2.95 million inpatients hospitalizations every year for people with OA and associated disorders[31]. While OA has been usually considered a non-life-threatening condition by both health care providers and patients[32], its association with cardiovascular diseases and diabetes results in an increased risk of death[33,34]. OA also represents a huge societal burden: in fact, people with OA are at greater risk for depression because of increased disability associated with their condition[35], and social isolation and loneliness are common among people with OA[36]. Workers with OA miss an average of two more days/year compared to colleagues without OA due to both absenteeism (absence from work) and presenteeism (reduced productivity at work)[37].

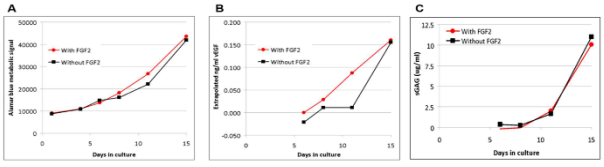
JTL proposes a new cure for OA, based on the injection of autologous adipose-derived stromal cells isolated at the bedside inthe knee joint. The innovation proposed by JTL resides on the unique combination of two components: i) the Mini-Stem System™,a unique portable and disposable closed system for the preparation and harvest of Autologous Adipose-Derived Stem Cells (ADSCs) from the Stromal Vascular Fraction (SVF) in non-controlled environments; ii) the RegenoGel carrier, the first clinically-approved viscosupplement product that can act as a scaffold to support stem cells growth, together with the protocol for ADSCs extraction, theircombination with RegenoGel and the administration to the patient.
The Mini-Stem System™ is designed to allow the transfer of tissue and fluid elements within a closed sterile pathway made of a combination of sterile containers and tubing (Figure 2). The RegenoGel carrier combines Hyaluronic Acid and Fibrinogen to provide long-lasting viscosupplement (up to one year) and support efficient delivery and maintenance of regenerative ADSCs for OA treatment. After injection into patients, the Regenogelscaffold will allow accelerated cell growth and release of cellular factors from the encapsulated stem cells to surrounding tissues to achieve desired joint healing in OA. At the same time, the hydrogel will provide additionallubrication for pain relief, creating a favorable environment for therapeutic effect.

JTL removes the technical barriers that prevent the generation of reliable and cost-effective clinical protocols for OA. The JTL Mini-Stem System™ will allow doctors instant access to autologous regenerative cell therapies, enabling POC treatment of OA in virtually any clinical setting. The closed-loop bedside process ofJTL Mini-Stem System™ allows bypassing the necessity for a controlled environment, highly skilled labpersonnel, and corresponding regulations. JTL will improve the clinical outcome as well as patients' satisfaction.The established method of ADSCs isolation will remove human errors to increase the quality and consistency ofcare. At the same time, JTL will help reduce or postpone the need for surgical intervention, helping to achievehealthcare cost savings. The competitive price proposed for the JTL therapeutic protocol ($5,000) will obliteratethe competition from available solutions designed for SVF isolation (e.g., Celution System from Plus Therapeutics, $150-200K), enabling quick market penetration.

The Mini-Stem System™ isolates ADSCs with a Chondroprotective effect. JTL extensively validated the ability of the Mini-Stem System™ to harvest autologous MSCs from adipose tissues through collaboration with scientists at Stanford University, and the University of South California. In collaboration with Roche Diagnostics USA, JTL developed and optimized a 2-hour protocol for adipose tissue[62] thatcomplies with the FDA definition of "minimal manipulative" cells. Compared to the traditional lab protocols, involving extensive manual labor in the controlled laboratory environment, the Mini-Stem System™ was confirmed to generate twice as many MSCs (Figure 3A). The system allowsto process up to 150 mL of lipoaspirate and obtains an averageyield of $0.6x10^6$ viable mononuclear cells every gram of fat, comprising 16% of MSCs positive cells (CD73+, CD90+, CD34+/CD45-). JTL verified that the ability of isolated cells to differentiate in chondrocytes (Figure 3B) and their ability to release Glycosaminoglycan (GAGs) with potential chondroprotective effect on the joints of patients suffering from OA. The therapeutic potential of SVC isolated with the Mini-Stem System™ has been confirmed in a small clinicalstudy. Patients affected by knee OA (n=47) were treated with an articular injection of an average of 40 million cells isolated with the Mini-Stem System™. More than 90% of patients treated expressed satisfaction with the treatment reporting pain reduction, significant functional improvement, and no adverse effects.

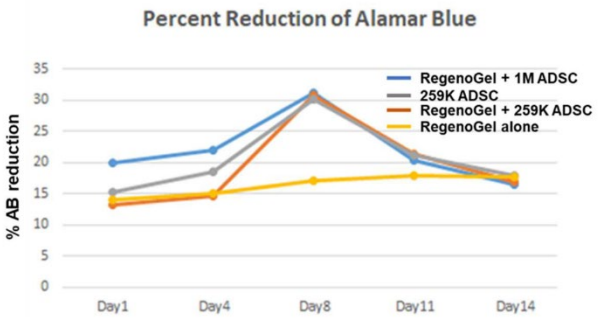


RegenoGel functionally supports cells isolated with Mini-Stem System™. *In vitro* RegenoGel has been shown to facilitate the growth and differentiation of a variety of human tissue-derived cells including chondrocytes and human adult stem cells[62]. JTL performed an early proof of concept study to verify that RegenoGel could beused as a scaffold for cells isolated with the Mini-Stem System™. The data showed excellent proliferation of cells embedded within the hydrogel and incubated in vitro (Figure 4A), which was reflected in gradually increased cell secretory activity. At the same time, RegenoGel remained fully intact after 15 days in culture, with no signs of degradation. A sharp increase in VEGF (Vascular endothelial growth factor) levels produced by the embedded cells was identified, indicating good survival and retention of MSCs potency within the hydrogel for at least 15 days (Figure 4B). The rise in the levels of soluble GAGs secreted by embedded cells is detectable after one week in culture, indicating the ability of RegenoGel to support the chondroprotective effect of the ADSCs.

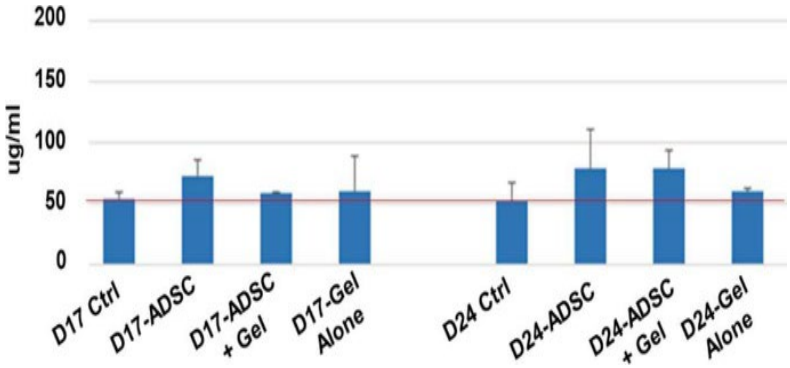


Phase I results

Aim 1: Optimization of ADSC-RegenoGel formulation for OA treatment.



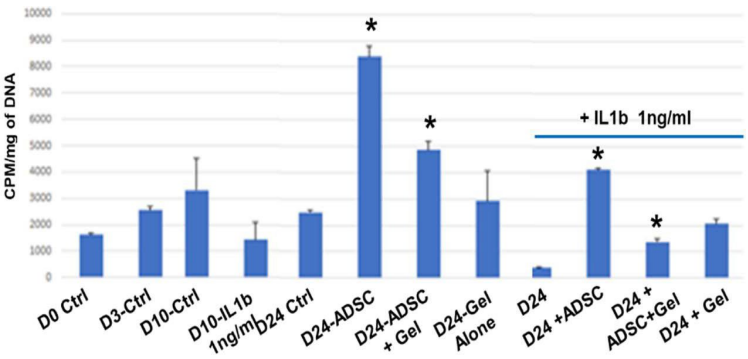
Task 1. RegenoGel supports the growth of ADSCs. Different concentrations of mononuclear cells harvested with Mini-Stem System™ were encapsulated into the RegenoGel matrix to establish an ADSC-RegenoGel formulation able to sustain optimal regenerative potential. ADSCs preparation was performed according to JTL protocol using the Mini-Stem System™. Viable mononuclear cells were counted and encapsulated into 1 mL of RegenoGel Matrix. Experiments were carried out with 259,000 and 1 million ADSCs, respectively. Four different conditions were tested as shown in Figure 5. Cell viability was measured by Alamar Blue staining (AB). All the samples (apart from the control, yellow line only RegenoGel) showed growth inhibition of approximately 69% eight days after seeding. This indicates that the culture had reached the saturation point and triggered reversed apoptosis and degeneration due to inadequate surface available for expansion. However, observation of the growth curve from Day 0 to Day 8 showed that the gel encapsulation provides adequate cell expansion support at both high (1 million) and low (250,000) ADSC concentrations. The concentration of cells for the Aim 2 experiments was determined based on the feasibility ofcell harvesting methodology related to Mini-Stem System™ to generate a number of cells at PoC. JTL assumedthat processing 50 mL of adipose tissue would deliver an estimated 50 million ADSC available for injection. Extrapolation of the knee joint cartilage surface to the surface of the culture dish led to the determination of a working concentration of 2 million cells/well.



Task 2. ADSCs stimulate Proteoglycan (PG) synthesis. PG synthesis by ADSC alone and gel-embedded ADSC was assessed during a 24-day culture period (Figure 6). The media of each experimental groupwere collected on Days 3, 10, 17, and 24. PG content was measured by Dimethyl Methylene Blue (DMMB) assay. Significant increases in PG content over the baseline of control (no cells), wereobserved both on days 17 and 24. The nominal value of PG was an avg. 17 µg/mL increase (32%, P=0.0015). It was impossible to reflect accumulated nominal values over time since the media had to be replaced every 48 hours. Yet, the values of days 17 and 24 are similar, which evidences the continuous release of GAGs by ADSC. Interestingly, GAG release by ADSCs was higher than that of gel-embedded ADSCs on day 17, while similar levels were observed on day 24.

Aim 2: Ex-vivo culture of articular cartilage with Adipose Stem Cells (ADSCs) / Hydrogel (Gel)

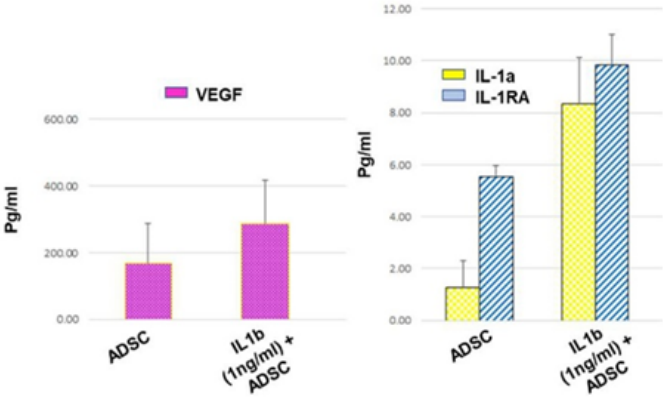
Articular cartilage samples were collected from ankle joints of human organ donors with no documented historyof joint diseases through the Gift of Hope Organ & Tissue Donor Network (Itasca, IL) within 24 hours of death. Full-thickness 4 mm cartilage explants were kept in a steady state for three days to adjust to culture conditions,after which the tissue was treated with 1ng/mL of IL-1β to mimic an inflammatory environment of the joint. After7 days of IL-1β treatment, ADSCs/Gel was added to their respective groups and cultured for 14 more days in the presence of MesenPro media. The media was replaced every other day. Cartilage samples and media werecollected by study design on days 3, 10, 17, and 24.



Quantification of PG synthesis in cartilage explants. PG synthesis in cartilage explants was measured on days 0 (baseline), 3, 10, and 24 of culture. The concentration of ^{35}S-PGs synthesized was normalized to DNA content measured by DNA assay using Hoechst 33258 dye. The effect of ADSCs on chondrocyte metabolism resulted in PG synthesis (Figure 7): ADSCs alone or when embedded in hydrogels were able to induce/elevate PG synthesis above culture control levels (p=0.033, D24_ADSC, and p=0,036 D24ADSC + gel respectively). Moreover, ADSC was able to restore PG synthesis inhibited by IL-1β. This effect reached statistical significance on day 24 of culture and was evident for both ADSCs alone and when ADSCs were embedded in hydrogel (p=0.0058 D24_ADSC+IL1β and p=0.004 ADSC + gel respectively). Interestingly, PG synthesis in groups containing ADSCs was not only higher than in the IL-1β control, but also higher than in any other controls (p<0.05). This indicates that ADSCs have indeed been able to affect the metabolism of articular chondrocytes in a pro-inflammatory environment. IL-1β induced inhibition was statistically significant when compared to culture control (MesenPro media, p=0.009) suggesting that IL-1 β was able to induce a pro-inflammatory response.

PCR for cartilage-specific genes: q-RT-PCR for cartilage-specific genes showed that COMP (Cartilage Oligomeric Matrix Protein) expression was significantly increased on Day 24 in the presence of ADSCs, while PTGS2 (often elevated in the synovial fluid and cartilage from patients with OA) and Sox9 showed a decrease in the presence of hydrogel embedded ASCs (data not shown). This effect by itself cannot be attributed to the increase of inflammation but rather to the complex effect of the ADSC-released cytokines on the modulation of the inflammatory process.

Analysis of Pro-inflammatory cytokines released into the culture media: Luminex xMAP technology (Luminex™



200 system, Luminex, Austin, TX, USA) was used for multiplexed quantification of 15 Human cytokines, chemokines, and growth factors. Fifteen markers, i.e. GM- CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-13, MCP-1 and TNF-α, were simultaneously measured in culture media using Eve Technologies' Human Cytokine Proinflammatory Focused 15- Plex Discovery Assay® (Millipore Sigma, Burlington, Massachusetts, USA). An increase in VEGF and IL-1RA levels was observed in presence of ADSCs (Figure 8), in response to inflammatory injury caused by IL-1. Other inflammation modulatory cytokines were modulated (data notshown) indicating a paracrine mechanism of action of ADSC when delivered into an inflammatory environment.

Conclusions

We found that gel encapsulation provided an adequate support for ADSCs expansion at both high (1 million) and low (250,000) concentrations and that ADSCs survived pro-inflammatory environment. The striking effect of ADSCs on chondrocyte metabolism was identified with PG synthesis. ADSCs alone or embedded in hydrogel induced/elevated PG synthesis above culture control levels (p=0.033 and p=0,036 respectively) and restored PG synthesis inhibited by IL-1β (p=0.058 and p=0.004

respectively). ADSCs also elevated an IL-1RA/IL-1 ratio by 4-fold alone or 7-fold in hydrogels in comparison to IL-1β control ($p<0.001$).

This study demonstrated that ADSC exhibited 1) a pro-anabolic activity by restoring and stimulating above control levels PG synthesis inhibited by IL-1β and 2) a continuous anti-inflammatory response by upregulating IL-1RA and its ratio to IL-1β. This is the first time when ADSCs were shown to engage in a complex pro and anti-inflammatory paracrine interaction with human adult chondrocytes in vitro. The results of this study suggest that ADSC survive an inflammatory environment, generate anti-inflammatory effect and restore synthesis of cartilage extracellular matrix components.

Summary:

1) **Regenogel supports the proliferation of ADSCs.**
2) **ADSCs (both alone or embedded in hydrogel) restore PG synthesis inhibited by pro-inflammatory IL- 1β both in culture media and cartilage explants.**
3) **ADSCs stimulate the production of pro- and anti-inflammatory cytokines, like VEGF and IL-1RA, correspondingly.**
4) **The trigger of proinflammatory factors leads to apparent anti-inflammatory effect, which accelerates in the weeks following the administration.**