APPLICATION FOR TECHNOLOGY TRANSFER

RABBIT ADIPOSE DERIVED MESENCHYMAL STEM CELLS SEEDED ON *TINOSPORA CORDIFOLIA* INCORPORATED DECELLULARIZED TENDON SCAFFOLD

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Name of the invention: Aqueous extract of *Tinospora cordifolia* incorporated decellularized tendon graft

Patent Application & priority date / Patent Number & date of patent:

Patent File number - 201941053464

Brief description of the invention (Abstract):
(1-2 sentences)

Tendons are often injured due to mechanical forces, with slow regeneration leading to weak scar tissue.
ADMSCs incorporated, DT scaffolds with Aqueous extract of *Tinospora cordifolia* in tissue-engineered scaffolds offers a promising, cost-effective, and accessible therapy for tendon injuries, showing potential for enhancing regeneration.

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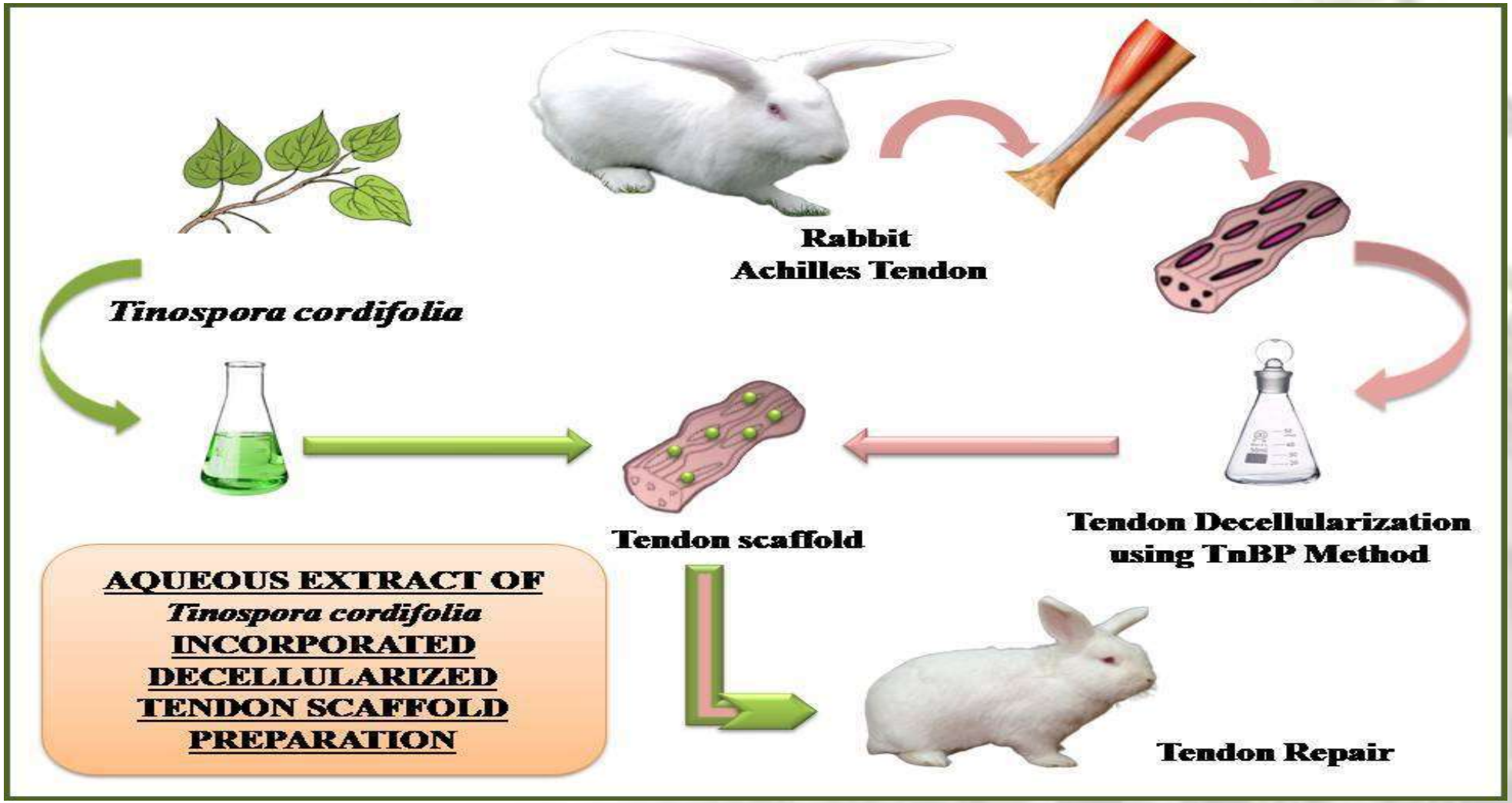
Graphical abstract:



Novelty of the invention:

Regeneration of tendon is slow due to its avascular nature and low cell proliferation. Healing results in scar tissue that is mechanically poor and thus prone to re-injury. Decellularization is a process that removes cells and cellular components, retaining biomechanical properties.

The surrogate extracellular matrix from decellularized tendon incorporated with aqueous extract of *Tinospora cordifolia* will form the tissue construct for tendon Tissue Engineering to enable regeneration and repair of worn-out/damaged/injured tendon for athletes and the geriatric community. This is a strategy to increase the efficacy and specificity of clinical procedures in tendon defect healing.

Natural biomimetic DT scaffolds were successfully prepared and evaluated the healing responses in tendon defect model by the creation of a nontoxic, biocompatible, easy to use and suturable Tissue Engineered Medical Product (TEMP) for tendon TE applications - (Novelty) an unmet clinical need in the orthopedic reconstructive surgeries and in turn a boon to sports/geriatric populations. This TE constructs combining traditional knowledge with cutting-edge technology - is a ‘MAKE IN INDIA’ concept with ‘BENCH TO BEDSIDE’ potential….

Utility of the invention:

RELEVANCE TO THE SOCIETY

- The restoration of the normal structure and function of the injured/damaged tendon represents one of the most challenging areas in regenerative medicine.
- Proposed product will **replace current systems that involve expensive imported synthetic polymeric grafts** and can be used as a platform for generating specific DT tissue in the long run.
- **An unmet clinical need in the orthopedic reconstructive surgeries…**

Society seeks an inexpensive method for the replacement of injured/damaged tendon for athletes, people in labor intensive occupation, geriatric population etc. — a boon to those are suffering from severe tendon related injuries!

Non-obvious nature of the invention:

- The invention described involves using a combination of extracellular matrix (ECM) derived from decellularized tendon, prepared using the Tri (n-butyl) phosphate procedure, and an aqueous extract of *Tinospora cordifolia* to heal damaged or worn-out tendons.

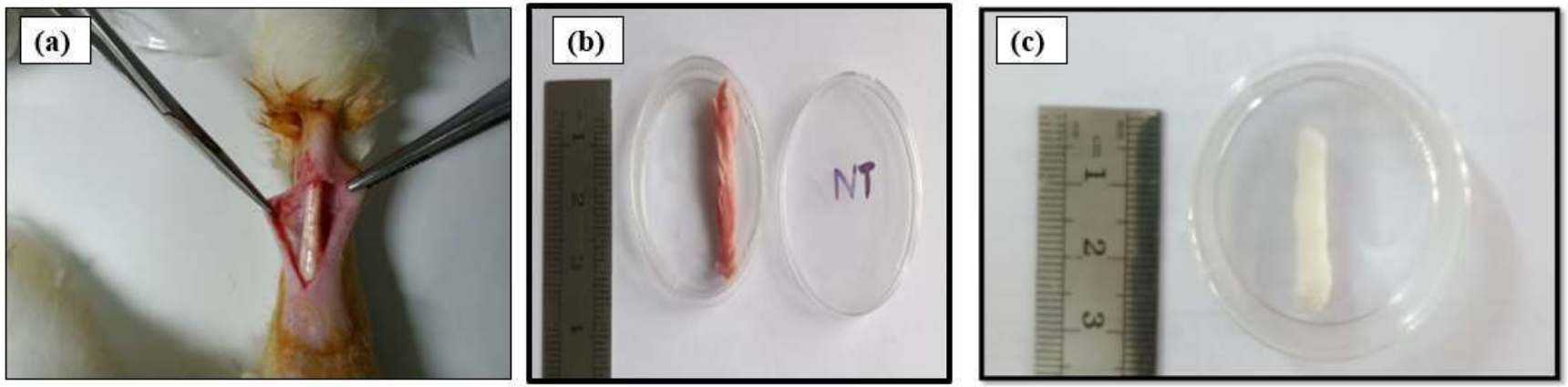
- This approach is significant because it represents a new generation of therapeutics that harness the benefits of plant extracts in tissue engineering.

- The decellularization process was successful in removing cells and preserving the ECM structure and biomechanical properties of the tendon, making it noncytotoxic and compatible with cells in vitro, and not eliciting an immune response in vivo.

- This comprehensive approach, combining innovative scaffold design with suitable cell sources and therapeutic phytochemicals, represents an ideal strategy for tendon repair, with no prior research addressing the simultaneous consideration of decellularization efficiency, preservation of native ultrastructure, mechanical properties, and ECM biochemical composition in tendon ECM scaffold preparation.

Results: (proof for clause 1)
Basic data only

Normal rabbit achilles tendon tissue | **Decellularized tendon scaffold**



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Results: (proof for clause 2)

MATERIAL DEVELOPMENT USING MODIFIED DECELLUARIZATION PROCESS




Saline wash – 3 times (10 mins) → Freeze thaw – Liq N_2 – 3 times (2 mins) → Saline wash – 3 times (10 mins) → Tris EDTA Buffer (3 hrs)

Triton x 100 (24 hrs) → Triton x 100 +TBP+Tris EDTA (24 hrs) → De ionized water (48 hrs) → Isopropanol (24 hrs)

Saline wash (30 mins) → Frozen samples stored at – 80^{0}C

In **Regenerative medicine**, organs are decellularized to remove cellular components to extract **acellular ECM/Biomimetic natural scaffolds.**

- ✓ After freeze-thaw cycles, tendon tissues were incubated with Triton x -100, TBP etc.
- ✓ In between every step, tissues were **washed with hypotonic, hypertonic solutions to achieve cell lysis.**

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Results: (proof for clause 3)

In vivo rabbit tendon defect model creation

Decellularized tendon construct is implanted into the Rabbit tendon defect model.

Group I Control

Group II DT

Group III RADMSC+DT

Group IV RADMSC+ DT+TC

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Results: (proof for clause 4)

Combining the **biomimetic properties** of **surrogate Extra Cellular Matrix (ECM) from the decellularized tendon** with **regenerative potential** of **Mesenchymal Stem Cells** and **Tenogenicity** inducing **phytochemicals** together might be a promising solution for tendon defect healing….

1. **Decellularized Tendon (DT) scaffolds were successfully prepared and characterized with various physiochemical characterizations.**
2. ***Tinospora cordifolia* extract containing phytochemical constituents have a potential activity to enhance the tendon regeneration process.**
3. **RADMSCs proved their stemness and capable of differentiation. Differentiated cells showed a gradual increase in viability as the time of incubation increases.**
4. **TC incorporated cell seeded DT scaffolds supports cell adhesion and growth; affordable substitute for tendon – suitable for orthopedic reconstructive surgeries.**
5. **Preclinical *in vivo* studies on New Zealand white rabbit models also confirmed the *in vitro* results of the effect of TC on better healing of tendon repair…**

Clauses applied for /protected (for granted patents):
NA

Fields where the invention finds application:

Natural biomimetic DT scaffolds were successfully prepared and evaluated the healing responses in tendon defect model by the creation of a nontoxic, biocompatible, easy to use and suturable Tissue Engineered Medical Product (TEMP) for tendon TE applications-an unmet clinical need in the orthopedic reconstructive surgeries and in turn a boon to sports/geriatric populations. This TE constructs combining traditional knowledge with cutting-edge technology - is a ‘MAKE IN INDIA’ concept with ‘BENCH TO BEDSIDE’ potential….

Whether the work has been published: (Authors, year, title of publication, Journal name, volume, page no)

- Niveditha K, John A, Joseph J, Mini S, Vineeth CA, Swapna TS, Abraham A. Natural 3D Extra Cellular Matrix mimicking stem cells seeded decellularized scaffolds as a platform for tendon regeneration. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2023 Sep;111(9):1672-86. D**OI:** 10.1002/jbmr.b.35265.
- Niveditha K,CA V, Joseph J, John A, Abraham A. Mesenchymal stem cells seeded decellularized tendon scaffold for tissue engineering. Current Stem Cell Research & Therapy. 2021 Feb 1;16(2):155-64. DOI: 10.2174/1574888X15666200723123901.

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