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The process of bone formation is driven by these main elements:

- Osteoconduction
- Osteoinduction
- Osteogenesis
- Angiogenesis

An ideal bone graft substitute provides all the necessary elements of bone formation in a single allograft.

Autogenous bone graft includes the elements necessary to promote bone growth-scaffolding for osteoconduction, growth factors for osteoinduction and viable cells for osteogenesis. Ideally, a bone graft substitute should do the same.\(^1\)\(^2\) Traditionally, osteoconduction, osteoinduction and osteogenesis have been considered the most important elements for new bone formation. Moreover, it is widely known that angiogenesis also plays a very important role in bone repair.\(^3\) New blood vessel formation allows for the migration of cells and necessary nutrients to the site of injury. Studies have shown that inhibition or promotion of vessel formation in an injury model can impact bone healing and that health factors that negatively impact neovascularization (e.g. smoking or diabetes) can cause delayed fracture healing or result in non-unions.\(^4\)\(^5\)\(^6\)

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\(^1\) A. R. Vaccaro. The role of the osteoconductive scaffold in synthetic bone graft. Orthopedics 2002; 25(5): s571-s578
\(^3\) K.D. Hankenson. Angiogenesis in bone regeneration. Injury 2011; 42(6): 556-561
\(^6\) H. Lu, et al. Diabetes interferes with the bone formation by affecting the expression of transcription factors that regulate osteoblast differentiation. Endocrinology 2003; 144(1): 346-352
Stem Cells and Bone Repair

Stem cells play several roles in the healing response throughout our lifetime. They respond to local environmental cues in order to differentiate and replace damaged cells or rebuild injured tissue. They also serve a signaling role themselves.

- Stem cells play several roles in the healing response:
  > Respond to local environmental cues in order to differentiate and replace damaged cells or rebuild injured tissue.
  > Signal and recruit host cells to participate in the healing response.
  > Provide angiogenic stimuli.
- Autologous sources of stem cells can be variable and are dependent on the age and health of the patient.
- Cellular bone grafts, which contain a reliable source of viable stem cells, are an ideal alternative for traditional bone grafting.

A stem cell is a primitive cell that has the ability to self-renew and differentiate into multiple specialized cell types. These cells are able to divide and replenish other cells in the body throughout our life span. Stem cells remain uncommitted until specific signals induce them to differentiate into various tissue types.

Stem cells play an important role in the natural healing process. This is done by either differentiating into specialized tissues themselves, or signaling the necessary host cells to participate in the healing process.

Although autograft and bone marrow aspirate have traditionally been used as a source of live cells for bone grafting, this is not always a viable option. The concentration of stem cells from autologous sources is variable and dependent on the age and health of the patient, as well as the collection site. It is well documented in the literature that as we age, there is a natural decrease in the concentration of available stem cells. Because of this, cellular allogeneic bone grafts, which contain a reliable source of viable stem cells, are an ideal alternative for traditional bone grafting.

MAPC-Class Cells Differentiation Potential

MAPC-class cells have the ability to form any of the three germ layers: mesoderm, ectoderm and endoderm. (Diagram 1) Since the cells are multipotent in nature, they have the capability to differentiate along the chondrogenic, adipogenic or osteogenic lineage (mesoderm layer). MAPC-class cells were cultured under appropriate conditions for each lineage and stained with oil red O, alizarin red or toluidine blue to confirm differentiation along the respective lineages.

Figure 3. Immunocytochemistry of MAPC-class cells along each differentiation lineage. (A) MAPC-class cells cultured on a tissue culture plate (20x); (B) Oil red O stained positive for fat accumulation, indicative of adipogenic differentiation (20x); (C) Alizarin red stained positive for calcium deposits/mineralization, indicative of osteogenic differentiation (20x); (D) Toluidine blue stained positive for cell aggregate for cartilage, indicative of chondrogenic differentiation (5x).
MAPC-Class Cells Have the Ability to Form Any of the Three Germ Layers

Diagram 1. The diagram above depicts the three primary germ layers in vertebrates and illustrates examples of cell types that are found in each of the germ layers. A germ layer is a primary layer of cells that forms during embryogenesis.
MAPC-Class Cells Osteogenic Potential

MAPC-class cells have demonstrated osteogenic potential in vitro. The osteogenic potential of the cells was assayed by looking at the presence of alkaline phosphatase, as well as the presence of mineral deposits.

- Alkaline phosphatase is an early indicator of active osteoblasts.
- Positive fast blue staining indicates the presence of alkaline phosphatase activity.

Figure 4. Alkaline phosphatase expression by MAPC-class cells cultured in (A) control or (B) osteogenic media for eight days. Fast blue staining indicated the presence of alkaline phosphatase activity only in cells cultured in osteogenic media (B).

- Mineralization and calcium deposits are late markers of osteogenicity.
- Positive alizarin red staining indicative of MAPC-class cells producing calcium deposits.

Figure 5. Mineral production by MAPC-class cells cultured in (A) control or (B) osteogenic media for 21 days. Alizarin red staining indicated the presence of mineral deposits only in cells in osteogenic media (B).
MAPC-Class Cells Angiogenic Properties

MAPC-class cells exhibit angiogenic properties that promote new vessel formation in vitro and in vivo (in a mouse model*) to a greater extent than mesenchymal stem cells (MSCs).

Angiogenic Protein Production by MAPC-Class Cells

- MAPC-class cells secreted specific angiogenic cytokines (IL-8 and CXCL-5) at higher levels when compared to MSCs.

An enzyme-linked immunosorbent assay (ELISA) was used to quantify the production of specific angiogenic cytokines by MAPC-class cells. Levels of angiogenic cytokines secreted by mesenchymal stem cells (MSCs) were assayed as well and compared to those of MAPC-class cells. Quantified production levels for IL-8 were significantly higher for MAPC-class cells, (p<0.005) when compared to MSCs. Furthermore, CXCL5 production levels were substantial for MAPC-class cells but undetectable for MSCs.

*Performance data from animal studies may not be representative of performance in humans.
MAPC-Class Cells Induce Vessel Formation by Endothelial Cells in an In Vitro Tube Formation Assay

- MAPC-class cells produced angiogenic cytokines which influenced endothelial cells to form tube-like structures in a three-dimensional matrix.
- Tube formation in the samples treated with MAPC-class cells conditioned media appeared more dense and well defined than those treated with MSCs.

The addition of MAPC-class cells-conditioned media to endothelial cells in culture (Figure 7D) induced the appearance of a dense web-like structure, similar to that observed in the positive control group (Figure 7B). This finding is indicative of MAPC-class cells releasing crucial angiogenic growth factors that induce the formation of vessel-like structures by endothelial cells.\(^9\) The MAPC-class cell group had denser and more well-defined tube formations when compared to MSCs (Figure 7C).

In Vivo Evaluation (Mouse Model*) Comparing Blood Vessel Formation in TCP with MAPC-Class Cells vs. TCP Alone Resulted in:

- 131% increase in new blood vessel formation with the addition of MAPC-class cells to TCP cubes (compared to the TCP alone).
- 29% increase in new blood vessel formation with MAPC-class cells over scaffold seeded with MSCs.

Porous ceramic tri-calcium phosphate cubes were incubated with a suspension of MAPC-class cells or mesenchymal stem cells (MSCs). Cell-loaded cubes and cell-free cubes were implanted subcutaneously into NOD/SCID mice, harvested at seven weeks post-implantation, stained and histologically evaluated for bone deposition and vessel formation.

Histologically, new bone formation and abundant new blood vessel formation were noted in MAPC-class cell-loaded cubes. The histological scores associated with new bone formation were 32% for MAPC-class cell-loaded, 27% for MSC-loaded and 0% for cell-free cubes. Moreover, new blood vessel formation was quantified. Ceramic cubes loaded with MAPC-class cells resulted in significantly (p<0.05) more vessel formation in the implant when compared to that of cell-free cubes, namely a 131% increase. In addition, cubes loaded with MAPC-class cells resulted in significantly (p<0.01) greater new vasculature when compared to the MSC-loaded cubes, namely a 29% increase.

Figure 8. Masson’s trichrome histological images at 100x magnification showing remodeling of the (A) empty ceramic cubes; (B) MSC-loaded ceramic cubes and (C) MAPC-class cell-loaded ceramic cubes. Note the abundant vessel and new bone formation in the MAPC-class cell-loaded cubes. NB = new bone, TCP = tricalcium phosphate cube particles. Black arrows point to blood vessels.

*Performance data from animal studies may not be representative of performance in humans.
MAPC-class cells were added to individual wells of a 24-well culture plate containing a transwell insert either with or without 50 mg of demineralized bone matrix (DBM; 2 lots). A cell-free group containing only an insert of DBM was also prepared. All groups were cultured in triplicate in osteogenic media for 14 days. VEGF was measured using an ELISA assay from supernatants collected after various time points.

VEGF release was low for the DBM alone sample. MAPC-class cells produced high levels of VEGF independently; however, when they were cultured with the DBM scaffold, the VEGF levels increased synergistically.

MAPC-class cells produced VEGF when cultured on a culture plate.

The amount of VEGF produced increased when the MAPC-class cells were cultured with DBM.

Figure 9. Vascular Endothelial Growth Factor (VEGF) protein release over a course of 14 days as assayed via ELISA. MAPC-class cells produced high quantities of VEGF. When MAPC-class cells were cultured in close proximity to DBM, VEGF production was significantly increased.
MAPC-Class Cells Immunomodulatory Properties

The natural healing process begins with inflammation and is followed by regeneration and remodeling. Inflammation is a result of the immune reaction that is initiated with any infection or injury. Although inflammation is a critical component of regeneration, a prolonged inflammatory response can have negative effects on the rate and quality of healing and lead to the formation of fibrotic or scar tissue. MAPC-class cells have been shown to affect both the inflammatory process as well as the immune response in specific ways.

**Attenuated Inflammatory Response**
- MAPC-class cells have shown potential for reducing local inflammation by regulating the production of inflammatory cytokines.
- Expression of the pro-inflammatory cytokine TNF-α was downregulated in the presence of MAPC-class cells conditioned media.

**Figure 10.** Lipopolysaccharides (LPS) were used to activate white blood cells (THP-1) which in response secrete pro-inflammatory signals such as TNF-α. MAPC-class cell-conditioned media contains signals that attenuate the inflammatory response and reduce the production of TNF-α by the activated white blood cells.

To study the immunomodulatory properties of MAPC-class cells, THP-1 cells were activated and induced to produce the inflammatory cytokine TNF-α. An ELISA assay was used to quantify TNF-α production levels. THP-1 cells alone had a heightened immune response as evidenced by the increased production of TNF-α. The experimental group, in which the THP-1 cells were cultured in MAPC-class cell-conditioned media (MCM), exhibited a reduction in the production of TNF-α.
Following pro-inflammatory signals, white blood cells, such as T-cells, get activated and recruited to the injury site to participate in the immune response. MAPC-class cells exhibited immunomodulatory functions and were able to suppress activated T-cell proliferation in vitro. MAPC-class cells have been shown to be non-immunogenic.

Peripheral blood mononuclear cells (PBMCs) were cultured in a mixed lymphocyte reaction (MLR) assay. PBMCs alone did not elicit a T-cell response. Stimulatory cytokines (CD3/CD28), which serve as signals to trigger an immune response, were used to activate PBMC cultures. Activated PBMCs showed an increase in the amount of viable T-cells as evidenced by the high thymidine incorporation counts indicative of active T-cell proliferation. Activated PBMCs co-cultured with MAPC-class cells exhibited a significant reduction in T-cell proliferation.

By modulating inflammatory signals and the proliferation of active immune cells, the immune response may be diminished, and the regeneration process can begin more promptly.

**MAPC-class cells have been demonstrated to be non-immunogenic.**

MAPC-class cells do not express MHC class II antigens and should not be recognized as foreign by the immune system.

- Isolation of the MAPC-class cells selects against hematopoietic cells and other cells that express HLA antigens and could cause an immune response. Testing is performed on every lot to ensure the absence of these contaminating cells.

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**Immunomodulatory Properties**

- MAPC-class cells have been shown not to elicit an immune rejection response and attenuated the proliferation of T-cells.

**Figure 11.** Activated peripheral blood mononuclear cells (PBMCs) cultured with MAPC-class cells. Thymidine incorporation counts were used to determine the rate of actively proliferating T-cells. MAPC-class cells have been shown not to elicit an immune response and attenuated the proliferation of T-cells in an activated PBMC sample.
Pre-Clinical Data: In Vivo Bone Formation

In Vivo Osteogenicity Model*

Methods:
- Implanted subcutaneously into NOD/SCID mice.
- Seven-week duration.
- Treatment groups:
  > TCP cubes alone or in combination with MAPC-class cells.

Results:
- New bone formation was evident in all of the MAPC-loaded cubes after seven weeks.
- No new bone matrix detected in the cell-free cubes.

*Performance data from animal studies may not be representative of performance in humans.
Evaluation of Bone Formation in a Rat Posterolateral Fusion (Spinal Fusion) Model*

Methods:
- L4 and L5 posterolateral fusion in rats.
- Six-week duration.
- Treatment groups:
  > DBM** alone or in combination with MAPC-class cells.

Results:
- The MAPC-class cell-loaded DBM scaffold had a significantly (p<0.05) higher histologic score, more new bone and less fibrous tissue compared to the DBM scaffold alone.
- Bridging was achieved between L4-L5 after six weeks.

*Performance data from animal studies may not be representative of performance in humans.

**Rabbit DBM
Evaluation of Bone Formation in a Rabbit Posterolateral Fusion (Spinal Fusion) Model*

**Methods:**
- L4 and L5 posterolateral fusion in rabbits.
- Five week duration.
- Treatment groups:
  > DBM** in combination with MAPC-class cells compared to morselized autograft iliac crest.

![Image of histological images](image)

**Figure 14.** H&E stained histological images at 100x magnification showing implanted graft remodeling. Both grafts were actively involved in remodeling. However, there was more new bone (NB) and less fibrotic tissue (FT) noted in cell-loaded DBM group, (B) indicative of advanced remodeling.

NB = new bone  
RG = residual graft  
BM = bone marrow  
FT = fibrous tissue

Black arrows point to new blood vessels, blue arrows point to osteoblasts and green arrows point to osteoclasts.

**Results:**
- DBM loaded with MAPC-class cells had significantly (p<0.05) higher histologic score and less fibrous tissue than the autograft group.
- Bridging bone indicative of fusion was observed after five weeks.

**Performance data from animal studies may not be representative of performance in humans.**
Evaluation of Bone Formation in a Sheep Critical Sized Defect Model*
(Femur Critical Sized Defect)

Methods:
- Critical defect wound (15mm diameter x 15mm depth) in sheep.
- Six-week duration.
- Treatment groups:
  - TCP granules in combination with MAPC-class cells compared to morselized sheep autograft iliac crest.

Results:
- The area between the MAPC-class cell-loaded TCP granules was filled with new bone after six weeks.
- There was less bone and more fibrous tissue in the autograft treated defects.

*Performance data from animal studies may not be representative of performance in humans.
Designed by nature, supported by science™
map3® Cellular Allogeneic Bone Graft

A Natural and Safe Alternative to Autograft

1. Cortical-cancellous bone chips provide a three-dimensional **osteconductive** scaffold to support cellular infiltration and attachment

   *Figure 16. map3 Chips scaffold*

2. Demineralized bone matrix (DBM) demonstrates verified **osteoinductive** potential*

   *Figure 17. Histological image (H&E, 100x) of DBM assayed in vivo in an athymic nude rat assay and determined to have osteoinductive potential."

   **NB = new bone
   BM = bone marrow
   DBM = residual DBM**

3. Cryogenically preserved, viable multipotent adult progenitor (MAPC®)-class cells provide **osteogenic** and **angiogenic** signals to support bone healing process

   *Figure 18. Masson’s trichrome histological images (100x) showing remodeling of MAPC-class cell loaded ceramic cubes in a mouse model.**

   **NB = new bone
   TCP = tricalcium phosphate cube particles
   Black arrows point to blood vessels**

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* DBM or representative finished implant is either assayed in vivo in the modified athymic nude rat model for bone formation or in vitro for endogenous BMP-2 as a surrogate test marker for osteoinductive potential. Because the combination of various proteins is responsible for osteoinductive potential, DBM when assayed in vitro, is also screened for the presence of BMP-7. Findings from an in vitro assay or animal model are not necessarily predictive of human clinical results.

**Performance data from animal studies may not be representative of performance in humans.
A natural and safe alternative to autograft that provides a streamlined approach to bone grafting.

<table>
<thead>
<tr>
<th>A Natural and Safe Alternative to Autograft</th>
<th>Three (3) elements of bone formation in a single allograft.</th>
<th>Mineralized cortical cancellous bone chips provide an osteoconductive scaffold.</th>
<th>Demineralized bone matrix (DBM) demonstrates verified osteoinductive potential.</th>
<th>Cryogenically preserved viable multipotent adult progenitor (MAPC)-class cells provide proven osteogenic, and angiogenic signals to support the bone healing process.</th>
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<tbody>
<tr>
<td>Focus on Cell Viability</td>
<td>A minimum of 50,000 viable MAPC-class cells/cc of implant are present on the scaffold at the time of implantation.</td>
<td>Donors are carefully selected for optimal cell quality.</td>
<td>Cell viability is confirmed at multiple points during the isolation process and validated for the graft's shelf life.</td>
<td>Recovery and isolation processes are designed to ensure optimal cell viability.</td>
</tr>
<tr>
<td>Easy to Use</td>
<td>Simple and straightforward preparation steps.</td>
<td>Multiple configurations and sizes provide unique handling characteristics and versatility.</td>
<td>Strips Allograft: Comprised of a three-dimensional scaffold, DBM and cryogenically preserved MAPC-class cells. Moldable implant with flexible yet cohesive properties making it ideal for filling bone voids in applications such as small joint repair, irregular defects or as an onlay in the posterolateral spine.</td>
<td>Chips Allograft: Comprised of DBM, cortical cancellous bone chips and cryogenically preserved MAPC-class cells. Available for applications such as filling bony defects and packing cages.</td>
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</table>
In order to provide a reliable minimum number of viable cells at implantation the viability losses due to cryopreservation, transportation and end user preparation of the cells must be understood and minimized. Starting with quantifying viable cells at isolation, cell viability measurements taken after the preservation, transportation, storage and implant preparation processes were used to determine a specific vialing concentration which ensures that each map3 allograft provides the targeted number of viable stem cells: 50,000 MAPC-class cells/cc of implant are present on the scaffold at the time of implantation.

In vivo cell tracking
MAPC-class cells are still present at the surgical site for at least 10 days after implantation.

*Performance data from animal studies may not be representative of performance in humans.*
Implant cell component safety is ensured through lot release testing which includes:

- Sterility testing based on methods outlined in USP <71>.
- Endotoxin testing based on methods outlined in USP <85>.
- Mycoplasma testing based on methods outlined in USP <63>.

Implant scaffold components are sterilized through the BioCleanse® Tissue Sterilization Process or Cancelle® SP DBM Sterilization Process as indicated. Where indicated sterile, processes applied achieve a Sterility Assurance Level of $10^{-6}$ via terminal sterilization.

Donor qualification is a rigorous, multi-step process that includes screening, testing and final determination of donor eligibility. The final determination of donor eligibility is made by RTI Surgical’s medical director — a licensed physician — utilizing all available, relevant information.

Donor infectious disease testing includes but is not limited to:

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<tr>
<td>HCV Antibody</td>
<td>Syphilis</td>
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<tr>
<td>HBV Surface Antigen</td>
<td>HTLV I &amp; II Antibody</td>
</tr>
<tr>
<td>HIV I &amp; II Antibody</td>
<td>HIV-1/NAT</td>
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<tr>
<td></td>
<td>HCV/NAT</td>
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<tr>
<td>HBV Total Core Antibody</td>
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Studies have shown that animals injected with MAPC-class cells did not develop tumors and did not show heterotopic bone formation.*

Map3 cellular allogeneic bone grafts have passed biocompatibility testing and are non-immunogenic, non-tumorigenic and safe.

*Data on file at RTI Surgical, Inc.
map3® cellular allogeneic bone graft is available in multiple configurations and a variety of sizes.

**map3® Strips Allograft**
The Strips Allograft is comprised of a three-dimensional scaffold, DBM and cryogenically preserved MAPC-class cells.

**Map3** Strips are available as a 2cc strip, 5cc strip and 10cc skinny strip. The 10cc size includes two strips per package.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tr>
<td>M3S002</td>
<td>map3® Strips Allograft, 2cc, 15 x 15 x 8 mm</td>
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<tr>
<td>M3S005</td>
<td>map3® Strips Allograft, 5cc, 25 x 25 x 8 mm</td>
</tr>
<tr>
<td>M3S010</td>
<td>map3® Strips Allograft, 10cc, 50 x 15 x 7 mm (2 each)</td>
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**map3® Chips Allograft**
The Chips Allograft is comprised of cortical cancellous bone chips, demineralized bone matrix and cryogenically preserved MAPC-class cells.

**Map3** Chips are available in three sizes: 2cc, 5cc and 10cc.

<table>
<thead>
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<tbody>
<tr>
<td>M3C002</td>
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<tr>
<td>M3C005</td>
<td>map3® Chips Allograft, 5cc</td>
</tr>
<tr>
<td>M3C010</td>
<td>map3® Chips Allograft, 10cc</td>
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RTI Surgical provides a focused team of product managers and biologics representatives for your support. Our dedicated customer service team is available 24 hours a day, 7 days a week, 365 days a year.

To order, call RTI Customer Service Sports & Extremities:

**800.624.7238**

Spine:

**888.778.8771**