Dental Stem Cells and Their Sources

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KEYWORDS

- Dental pulp stem cells (DPSCs)
- Stem cells from human exfoliated deciduous teeth (SHED cells)
- Stem cells from root apical papilla (SCAP cells)
- Periodontal ligament stem cells (PDLSCs) • Dental follicle precursor cells (DFPCs)

KEY POINTS

- The search for more accessible mesenchymal stem cells than those found in bone marrow has propelled interest in dental tissues, which are rich sources of stem cells. Human dental stem/progenitor cells (collectively termed dental stem cells [DSCs]) that have been isolated and characterized include dental pulp stem cells, stem cells from exfoliated deciduous teeth, stem cells from apical papilla, periodontal ligament stem cells, and dental follicle progenitor cells.
- The common characteristics of these cell populations are the capacity for self-renewal and the ability to differentiate into multiple lineages (multipotency). In vitro and animal studies have shown that DSCs can differentiate into osseous, odontogenic, adipose, endothelial, and neural-like tissues.
- In recent studies, third molar dental pulp somatic cells have been reprogrammed to become induced pluripotent stem cells, and dental pulp pluripotentlike stem cells have been isolated from the pulps of third molar teeth.

INTRODUCTION

The aim of regenerative medicine and tissue engineering is to replace or regenerate human cells, tissue or organs, to restore or establish normal function.[1] The 3 key elements for tissue engineering are stem cells, scaffolds, and growth factors. Cell-based therapies are integral components of regenerative medicine that exploit the

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inherent ability of stem cells to differentiate into specific cell types. The extension of basic stem cell science into translational therapies is already well established with artificial skin therapies,[2] whereas research is ongoing for cell-based therapies to target other diseases, including diabetes,[2] atherosclerosis,[3] and neurodegenerative diseases.[4] The search for more accessible mesenchymal stem cells (MSCs) than those found in bone marrow has propelled interest in dental tissues, which are rich sources of stem cells. This article provides an overview of stems cells and then focuses on dental stem cells (DSCs) and how recent developments have the potential to greatly impact the way DSCs might be used in future regenerative medicine applications that include regenerative endodontic therapies.

STEM CELLS

General Characteristics

Stem cells are undifferentiated embryonic or adult cells that continuously divide. A fundamental property of stem cells is self-renewal or the ability to go through numerous cycles of cell division while maintaining the undifferentiated state (**Box 1**). In addition, stem cells produce intermediate cell types (called progenitor or precursor cells) that have the capacity to differentiate into different cell types and generate complex tissues and organs.[5] Differentiation occurs when a stem cell acquires the features of a specialized cell (eg, odontoblast).

Stem cells can be either embryonic or adult (postnatal). Thomson and colleagues[6] first reported human embryonic stem cell lines in 1998. Embryonic stem cells are isolated from the blastocyst during embryonic development and give rise to the 3 primary germ layers: ectoderm, endoderm, and mesoderm. These cells are totipotent or pluripotent with an unlimited capacity to differentiate and can develop into each of the more than 200 cell types of the adult body (**Box 2**).

Adult stem cells exist throughout the body in different tissues, including bone marrow, brain, blood vessels, liver, skin, retina, pancreas, peripheral blood, muscle, adipose tissue, and dental tissues. They are localized to specific niches where the regulation of stem cell proliferation, survival, migration, fate, and aging occur.[5,7] Whether cells undergo either prolonged self-renewal or differentiation depends on intrinsic signals modulated by extrinsic factors in the stem cell niche.[8] An adult stem cell can divide and create another cell like itself, and also a cell more differentiated than itself, but the capacity for differentiation into other cell types is limited. This capability is described as being multipotent and is a distinguishing feature of adult stem cells compared with the pluripotency of embryonic stem cells. Although early research suggested that adult stem cells were limited in the types of tissues they produced, it is increasingly apparent that adult stem cells have greater plasticity than previously thought and can generate a tissue different to the site from which they were originally isolated.[9,10] An example with potential clinical applications is the ability of dental pulp cells to generate heart tissue in rats.[11]

Box 1
Fundamental properties of stem cells

Undifferentiated cells	Have not developed into a specialized cell type
Long-term self-renewal	The ability to go through numerous cycles of cell division while maintaining the undifferentiated state
Production of progenitor cells	Capacity to differentiate into specialized cell types (eg, odontoblast, osteoblast, adipocyte, fibroblast)

Box 2 Stem cell potency		
Embryonic stem cells from inner cell mass of 3- to 5-day embryo (blastocyst)	Totipotent	Can give rise to all the cell types of the body, including those cells making the extraembryonic tissues (eg, placenta) Unlimited capacity to divide
Embryonic stem cells Induced pluripotent stem cells	Pluripotent	Can form derivatives of all the embryonic germ layers (ectoderm, mesoderm, and endoderm) from a single cell Can give rise to all of the various cell types of the body
Adult stem cells (postnatal)	Multipotent	Can give rise to more than one cell type of the body
Induced pluripotent stem cells	Pluripotent	Derived from somatic cells

MSCs

In 1963, hematopoietic stem cells giving rise to blood cells were identified in bone marrow.[12] Since then, it has been established that bone marrow is also the primary source for multipotent MSCs.[13] Bone marrow MSCs (BMMSCs) can differentiate into osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic lineages. MSCs are found in many other tissues in the body, including umbilical cord blood, adipose tissue, adult muscle, and dental tissues[13]; are capable of differentiating into at least 3 cell lineages: osteogenic, chondrogenic, and adipogenic[14]; and can also differentiate into other lineages, such as odontogenic, when grown in a defined microenvironment in vitro.[13,14]

Definitive information on the location and distribution of MSCs is still being elucidated. However, it has been shown that MSCs can be found around blood vessel walls and perineurium as demonstrated by the immuno-colocalization of STRO-1/CD146 stem cell markers.[15] These observations have led to the proposal that MSCs arise from a perivascular stem cell niche[15,16] that provides an environment allowing the cells to retain their stemness.[14,17] Crisan and colleagues[15] demonstrated that human perivascular cells from diverse and multiple human tissues give rise to multi-lineage progenitor cells that exhibit the features of MSCs. Perivascular progenitor/stem cells can also proliferate in response to odontoblast injury by cavity preparation under ex vivo tooth culture conditions.[18]

Isolation, Identification, and Differentiation of MSCs

A fundamental approach to isolate MSCs in tissue samples involves the enzymatic digestion of tissue followed by the growth of isolated cells (expansion) in medium rich in growth factors.[19,20] The isolation of more immature stem cells involves a multistep explant approach whereby pieces of tissue are first cultured until progenitor cells grow after which enzymatic digestion and expansion in media proceed.[7,21]

The identification of MSCs uses a series of in vitro tests. Colony-forming assays are used to confirm clonogenicity (the ability to generate identical stem cells with the appropriate cell morphology), which is a consistent characteristic of MSCs. Phenotypic assays evaluate cell morphology or shape (eg, fibroblastic when flat and elongated) and cell behavior (eg, secretory). The possession of one or several cell surface markers found on cells in representative tissues is evaluated by flow cytometry, which sorts cells with specific surface protein, such as STRO-1, found on stem cells that can differentiate into multiple mesenchymal lineages, including dental pulp

cells (**Fig. 1**C, F, I).[14,22] DSCs can also express specific proteins associated with endothelium (CD106, CD146), perivascular tissues (α-smooth muscle actin, CD146, 3G5), bone, dentin and cementum (bone morphogenic protein [BMP], alkaline phosphatase, osteonectin, osteopontin, and bone sialoprotein), and fibroblasts (type I and III collagen).[23,24]

In vitro functional assays test putative MSCs for multipotency by confirming that differentiated cells demonstrate the appropriate phenotypic characteristics. Accordingly, the in vitro confirmation of the multipotency of dental pulp stem cells (DPSCs) can be demonstrated by the evidence of odontoblastlike differentiation (verified by the deposition of mineralized matrix and positive staining for dentin sialophosphoprotein), adipogenic differentiation (by the accumulation of lipid vacuoles), chondrogenic differentiation (by the production of collagen type II), and neurogenic differentiation (by neuronal-cell morphologies and markers).[25–31]

In vivo functional assays are used to confirm that stem cells implanted into a new environment (eg, immunodeficient mice) successfully integrate with adjacent cells, survive, and function as differentiated cells.[32] Several studies have demonstrated the formation of new pulp and dentinlike tissues following the insertion of DSCs

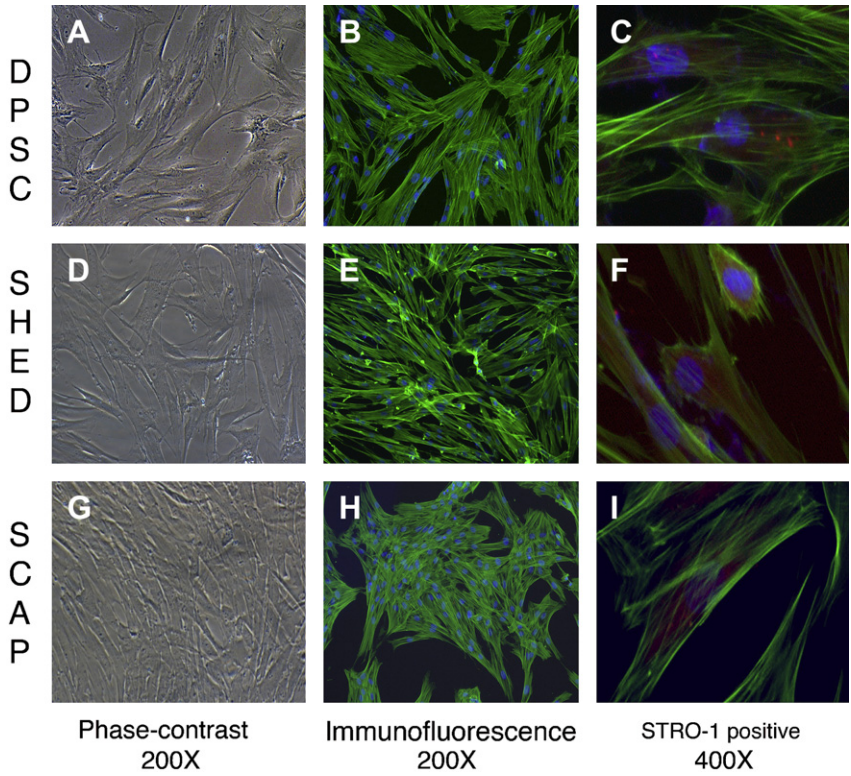

Fig. 1. Microphotographs showing morphology of stem cells in culture: DPSCs (*A*), SHED cells (*D*), and SCAP cells (*G*) (phase contrast, original magnification ×200). Microphotographs for cytoskeleton labeled for actin-F (*green fluorescent dye*), nuclei with DAPI (*blue fluorescent dye*), and STRO-1 positive cells (*red fluorescent dye*) for DPSCs (*B*, *C*), SHED cells (*E*, *F*), and SCAP cells (*H*, *I*) ([*B*, *E*, *H*] immunofluorescence, original magnification ×200; [*C*, *F*, *I*] STRO-1 positive, original magnification ×400). DPSC, dental pulp stem cells; SCAP, stem cells from apical papilla; SHED, stem cells from human exfoliated deciduous teeth.

seeded onto scaffolds in emptied human root canals or dentin disks embedded into immunocompromised mice; the resulting dentinogenesis is accomplished by odontoblastlike cells derived from MSCs.[16,23,27,28,31–34]

Storage of Stem Cells

Adult stem cells can be obtained from individuals at any stage in life and, therefore, can provide a source of cells for autologous transplants.[35] Such procedures invariably require stem cell storage, which is achieved by cryopreservation in liquid nitrogen (−196°C). Stem cells can survive these low temperatures as long as they are dispersed in cryprotectants.[36,37] Human periodontal ligament stem cells (PDLSCs) have been successfully recovered after cryopreservation for 6 months; although the number of colonies was less than for fresh PDLSCs, the proliferation rate was similar.[37] Similarly, stem cells isolated from human third molar teeth and cryopreserved for at least 1 month retained STRO-1 marker expression and the potential to proliferate into neurogenic, adipogenic, osteogenic/odontogenic, myogenic, and chondrogenic pathways in inductive media.[36] Cryopreservation of intact teeth provides another potential storage method that can allow later extraction of stem cells demonstrating similar behavior as stem cells extracted from fresh teeth.[38,39]

DSCs

A tooth develops as a result of carefully orchestrated interactions between the oral epithelial ectodermal cells that form the enamel organ (for enamel formation) and cranial neural crest–derived mesenchymal cells that form the dental papilla and dental follicle. These MSCs give rise to the other components of the tooth: dentin, pulp, cementum, and periodontal ligament. Beginning in 2000,[23] several human dental stem/progenitor cells have been isolated and characterized (**Box 3**). These cells include human DPSCs from permanent teeth (see **Fig. 1**A–C),[23] stem cells from exfoliated deciduous teeth (SHED cells) (see **Fig. 1**D–F),[25] stem cells from apical papilla (SCAP cells) (see **Fig. 1**G–I),[27] PDLSCs,[40] and dental follicle precursor, or progenitor, cells (DFPCs).[41] Although MSCs from different DSCs form distinct populations,[24] among their common characteristics are the capacity for self-renewal and the ability to differentiate into at least 3 distinct lineages.[14,24] The regeneration/revascularization of pulpal tissues uses DSCs in partnership with growth factors, scaffolds, and vascular supply.[32,42,43]

DPSCs

DPSCs were first isolated from human permanent third molars in 2000.[23] The cells were characterized as clonogenic and highly proliferative. Colony formation frequency was high and produced densely calcified, albeit sporadic, nodules.[23] Dentin and pulplike tissues were generated following the transplantation of DPSCs in

Box 3
Dental stem cells

DFPCs	Dental follicle precursor cells
DPPSCs	Dental pulp pluripotentlike stem cells
DPSCs	Dental pulp stem cells
DSCs	Dental stem cells
PDLSCs	Periodontal ligament stem cells
PDLPs	Periodontal ligament progenitor cells
SCAP cells	Stem cells from apical papilla
SHED cells	Stem cells from human exfoliated deciduous teeth

hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolds into immunodeficient mice.[23,24] A follow-up study confirmed that DPSCs fulfilled the criteria needed to be stem cells: an ability to differentiate into adipocytes and neural cells and odontoblasts (ie, multipotency) and self-renewal capabilities.[31] Additional studies have confirmed that DPSCs can also differentiate into osteoblast-, chondrocyte-, and myoblastlike cells and demonstrate axon guidance.[10,44–46]

It is now recognized that DSCs can play an important role in the balance of inflammation and repair/dentinogenesis during invasive caries lesions or pulp exposures.[47,48] Following odontoblast damage after caries or trauma, markers of inflammation and regeneration within the pulp tissue are differentially expressed,[47,49,50] with crosstalk between the inflammatory and regenerative processes considered to determine the outcome.[51] This notion is supported by in vitro observations of DPSCs migrating from the perivasculature toward the dentin surface following injury to the dentin matrix and differentiating into functional odontoblasts in response to EphB/ephrin signaling.[52,53] DPSCs have also been shown to express the bacterial recognition toll-like receptors, TLR4 and TLR2, and vascular endothelial growth factor in response to lipopolysaccharide, a product of gram-negative bacteria.[20,48,54] When compared with normal pulps, DPSCs in inflamed pulp tissues have reduced dentinogenesis activity,[55] and an in vitro investigation has shown reduced dentinogenic potential of DPSCs exposed to a high bacterial load that can be recovered after the inhibition of the bacterial recognition toll-like receptor TLR2.[48] Taken together, these studies support the existence of interactions between DSCs and immune cells in pulps affected by dental caries,[47] a better understanding of which has significant implications for the future management of teeth affected by dental caries.

In an effort to determine the fate of DPSCs exposed to root canal irrigants used in regenerative endodontic therapy procedures, dentin disks were preconditioned with different irrigants (5.25% sodium hypochlorite [NaOCl] or 17% ethylenediaminetetraacetic acid [EDTA]), seeded with DPSCs, and implanted subcutaneously into immunodeficient mice.[56] After 6 weeks, the differentiation of DPSCs into odontoblastlike cells was facilitated by the use of EDTA. In contrast, the use of NaOCl resulted in resorption lacunae at the cell-dentin interface.

SHED Cells

SHED cells are highly proliferative stem cells isolated from exfoliated deciduous teeth capable of differentiating into a variety of cell types, including osteoblasts, neural cells, adipocytes, and odontoblasts, and inducing dentin and bone formation.[25] Like DPSCs, SHED cells can generate dentin-pulplike tissues with distinct odontoblastlike cells lining the mineralized dentin-matrix generated in HA/TCP scaffolds implanted in immunodeficient mice.[24] However, SHED cells have a higher proliferation rate than DPSCs and BMMSCs, suggesting that they represent a more immature population of multipotent stem cells.[25,33,57] SHED cells have shown different gene expression profiles from DPSCs and BMMSCs; genes related to cell proliferation and extracellular matrix formation, such as transforming growth factor (TGF)-β, fibroblast growth factor (FGF)2, TGF-β2, collagen (Col) I, and Col III, are more highly expressed in SHED cells compared with DPSCs.[57]

In tissue engineering studies, odontoblastic and endothelial differentiation occurred when SHED cells were seeded in tooth slices/scaffold and implanted subcutaneously into immunodeficient mice.[33,34] The resultant tissues closely resembled those of human dental pulp, and tubular dentin mediated by dentin-derived BMP-2 protein was secreted.[33,58] These findings, together with those of other studies, suggest that SHED cells from exfoliated deciduous teeth may be an excellent resource for stem cell therapies, including autologous stem cell transplantation and tissue engineering.[7,25]

Regenerative endodontic therapy procedures should avoid compromising the attachment of stem cells to dentin. An in vitro study showed that the root canal irrigants 6% NaOCl and 2% chlorhexidine (CHX) were cytotoxic to SHED cells. In addition, the attachment of SHED cells to root canal dentin pretreated with NaOCl or CHX was reduced compared with negative controls (saline pretreatment).[59]

SCAP Cells

SCAP cells are found in the apical papilla located at the apices of developing teeth at the junction of the apical papilla and dental pulp.[27,60,61] The apical papilla is essential for root development. SCAP cells were first isolated in human root apical papilla collected from extracted human third molars.[27] The cells are clonogenic and can undergo odontoblastic/osteogenic, adipogenic, or neurogenic differentiation. Compared with DPSCs, SCAP cells show higher proliferation rates and greater expression of CD24, which is lost as SCAP cells differentiate and increase alkaline phosphate expression.[27,60,62]

SCAP cells seeded onto synthetic scaffolds consisting of poly-D,L-lactide/glycolide inserted into tooth fragments, and transplanted into immunodeficient mice, induced a pulplike tissue with well-established vascularity, and a continuous layer of dentinlike tissue was deposited onto the canal dentinal wall.[32] In a minipig, a bio-root was created by using autologous human SCAP cells seeded in an HA/TCP root-shaped carrier coated with Gelfoam (Pharmacia Canada Inc., Ontario, Canada) carrying PDLSCs that were implanted in the alveolar socket of a recent extracted anterior tooth.[27] After 4 months, the resulting bio-root was capable of supporting a porcelain crown and participating in normal tooth function.[27]

Root canal irrigants used in regenerative endodontic therapy procedures should ideally support cell survival, or at least not compromise survival. An in vitro study showed that 17% EDTA used alone supported SCAP cell survival better (89% survival) than when used with either 6% NaOCl (74% survival) or 2% CHX (0% survival).[63]

PDLSCs

McCulloch[64] reported the presence of progenitor/stem cells in the periodontal ligament of mice in 1985. Subsequently, the isolation and identification of multipotent MSCs in human periodontal ligaments were first reported in 2004.[40] Seo and colleagues[40] demonstrated the presence of clonogenic stem cells in enzymatically digested PDL and further showed that human PDLSCs transplanted into immunodeficient rodents generated a cementum/PDL-like structure that contributed to periodontal tissue repair. Later work showed that PDLSCs differentiation was promoted by Hertwig's epithelial root sheath cells in vitro.[65]

PDLSCs have the capability to differentiate into cementoblastlike cells, adipocytes, and fibroblasts that secrete collagen type I.[66] As with BMMSCs, PDLSCs can undergo osteogenic, adipogenic, and chondrogenic differentiation.[67] PDLSCs have also been shown to differentiate into neuronal precursors.[68] A recent retrospective pilot study showed evidence of the therapeutic potential of autologous periodontal ligament progenitor cells obtained from third molar teeth implanted on bone grafting material into intrabony defects in 2 patients.[69] After 32 to 70 months, a marked improvement was found in all sites. The progenitor cells behaved like PDLSCs, although they did not express the same markers.[69]

DFPCs

The dental follicle forms at the cap stage by ectomesenchymal progenitor cells. It is a loose vascular connective tissue that contains the developing tooth germ, and

progenitors for periodontal ligament cells, cementoblasts, and osteoblasts. DFPCs were first isolated from the dental follicle of human third molars.[41]

Because DFPCs come from developing tissue, it is considered that they might exhibit a greater plasticity than other DSCs.[70] Indeed, different cloned DFPC lines have demonstrated great heterogeneity.[71] In addition, after transplantation in immunodeficient rodents, DFPCs differentiated into cementoblastlike[72,73] and osteogeniclike[74] cells, and surface markers compatible with those of fibroblasts were identified in human dental follicle tissues, suggesting the presence of immature PDL fibroblasts.[75] DFPCs were able to differentiate into odontoblasts in vitro, and four weeks after combining rat DFPCs with treated dentin matrix the root-like tissues stained positive for markers of dental pulp.[26,76] Both DFPCs and SHED cells can differentiate into neural cells; however, these are differentially expressed when the cells are grown under the same culture conditions.[77]

Induced Pluripotent Stem Cells and Dental Pulp Pluripotentlike Stem Cells

In breakthrough studies in 2006 and 2007, investigators described methods to reprogram somatic cells from mice,[78] and subsequently humans,[79,80] by the insertion of 4 genes (*OCT3/4, SOX2, KLF4,* and *MYC*) that reprogrammed the somatic cells and returned them to an embryolike state. The resultant induced pluripotent stem (iPS) cells have embryonic stem cell characteristics: they are capable of generating cells from each of the 3 embryonic germ layers and can propagate in culture indefinitely. The pluripotency of human stem cells can be tested in vitro by the aggregation and generation of embryoid bodies from cultured cells[81] and in vivo by teratoma formation after cells are injected subcutaneously into immunodeficient mice.[82] The use of *MYC* is now avoided because it might induce malignant tumor formation and, therefore, would be contraindicated for clinical application.[83]

Recent reports have described successful attempts to develop pluripotent stem cells from pulps recovered from deciduous teeth[7] and from third molar pulps.[84,85] Indeed, deriving iPS cells from deciduous teeth DPSCs was reported to be easier and more efficient compared with human fibroblasts.[7] Oda and colleagues[84] reported successfully reprogramming mesenchymal stromal cells derived from the pulps of young human third molars (obtained from patients aged 10, 13, and 16 years) by retroviral transduction of the transcription factors *OCT3/4, SOX2,* and *KLF4*. The resultant cells had high iPS clonal efficiency suggesting a potential role for dental pulp stromal cells in regenerative medicine. A recent report describes the isolation of dental pulp pluripotentlike stem cells (DPPSCs) from the pulps of human third molar pulp tissue obtained from 20 patients of different genders and ages ranging from 14 to 60 years.[85] When the cells were injected into nude mice, teratomalike structures developed that contained tissues derived from all 3 embryonic germ layers. Significantly, the investigators noted that even in older patients, there was always a population of DPPSCs present.[85]

Although iPS cells are not truly equal to embryonic stem cells,[86] and may even have a memory of the somatic tissue from which they were derived,[87] they have generated great interest for their many potential personalized regenerative therapeutic applications.[88] For example, disease-causing mutations could be repaired by reprogramming. Another potential application is to use iPS cells derived from patients with diseases for drug development and in vitro disease modeling.[89,90]

SUMMARY

The ready availability of DSCs makes them a viable source of adult MSCs for regenerative medicine applications. Human dental stem/progenitor cells that have been

isolated and characterized include DPSCs, SHED cells, SCAP cells, PDLSCs, and DFPCs. Although much work is required for the translation of data from in vitro and animal studies to viable clinical applications, there are exciting possibilities for the use of DPSCs in tissue engineering and regenerative medicine applications within the root canal, the oral cavity, and in other parts of the body. Finally, the relative ease with which DSCs can be obtained, coupled with interest in stem cell banking,[91,92] will likely drive research that further elucidates their characteristics and potential applications.

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